(n=6), neuropathy (n=3), development of an unrelated malignancy (glioblastoma) (n=1), reduced-intensity allogeneic transplantation (n=1).

This is the first study to use internationally agreed response criteria for myelofibrosis (the EUMNET criteria) to evaluate response to thalidomide and prednisolone. Overall, 40% of patients achieved major or moderate responses by EUMNET criteria. All responses began within the first 12 weeks of treatment, suggesting that thalidomide could be stopped if there is no response by this time. Among responders, the median response duration was 16 weeks. Three patients lost their responses when prednisolone was withdrawn at 12 weeks. The 2 patients not treated with prednisolone appeared to have a less favourable response.

Our results are consistent with those of phase II trials.[4] In the Mesa trial,[4] 40% became transfusion independent, 75% had a >50% rise in platelet count, and 19% had a >50% reduction in spleen size. In the Marchetti trial,[5] 39% became transfusion independent, 22% had a >50x10^9/L rise in platelet count, and 19% had a >50% reduction in spleen size.

The relative importance of thalidomide and prednisolone when used in combination is unclear, but prednisolone seems to be partly responsible for responses. Case reports suggest that corticosteroids have efficacy as monotherapy in myelofibrosis.[9] However, our response rates are similar to those obtained with thalidomide monotherapy (median 100 mg/d). Three of our patients experienced a loss of clinical response when prednisolone was stopped consistent with other studies[4] and 2 patients not treated with prednisolone showed a poorer performance.

Our data confirms that low-dose thalidomide with prednisolone is effective treatment for myelofibrosis, leading to transfusion independence, improvement in thrombocytopenia and reduction in spleen size in some patients. We recommend assessment of thrombotic risk and thromboprophylaxis for high-risk patients. Low-dose thalidomide is not effective in all patients, and responses are often not sustained after withdrawal of prednisolone.

Robert Weinkove,[1] John T. Reilly,[2] Mary Frances McMullin,[3] Natasha J. Curtin,[4] Deepti Radia,[1] and Claire N. Harrison[1]

[1]Department of Haematology, St Thomas' Hospital, London; [2]Department of Haematology, Royal Hallamshire Hospital, Sheffield; [3]Department of Haematology, Belfast City Hospital, Belfast; [4]Department of Haematology Queen Elizabeth Hospital, Gayton Road, King's Lynn, UK

Acknowledgments: we wish to thank Drs. N. Ketley and J. Ropner for provision of patient data.

Correspondence: Claire Harrison, Department of Haematology, St Thomas' Hospital, Guy's and St Thomas' NHS Foundation Trust London SE1 7EH, UK. Phone: international +44.207.1882742. Fax: international +44.207.1882728. E-mail: claire.harrison@gstt.nhs.uk

Citation: Weinkove R, Reilly JT, McMullin MF, Curtin NJ, Radia D, Harrison CN. Low-dose thalidomide in myelofibrosis. Haematologica 2008; 93:1100-1101. doi: 10.3324/haematol.12416

References

1. Cervantes F. Modern management of myelofibrosis Br J Haematol 2005;128:583-92.
2. Mesa RA, Hanson CA, Rajkumar SV, Schroeder G, Tefferi A. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. Blood 2000;96:3374-80.
3. Elliott MA, Mesa RA, Li CY, Hook CC, Ansell SM, Levitt RM, et al. Thalidomide treatment in myelofibrosis with myeloid metaplasia. Br J Haematol 2002;117:288-96.
4. Mesa RA, Steensma DP, Pardanani A, Li CY, Elliott M, Kaufmann SH, et al. A phase 2 trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. Blood 2003; 101: 2534-41.
5. Marchetti M, Barosi G, Balestri F, Viarengo G, Gentili S, Barulli S, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial J Clin Oncol 2004;22:424-31.
6. Barosi G, Ambrosetti A, Finelli C, Grossi A, Leoni P, Liberato NL, et al. The Italian Consensus Conference on Diagnostic Criteria for Myelofibrosis with Myeloid Metaplasia. Br J Haematol 1999;104:730-7.
7. Barosi G, Bordessoule D, Briere J, Cervantes F, Demory JL, Dupriez B, et al. Response criteria for myelofibrosis with myeloid metaplasia: results of an initiative of the European Myelofibrosis Network (EUMNET). Blood 2005;106: 2849-53.
8. Tefferi A, Barosi G, Mesa RA, Cervantes F, Deeg HJ, Reilly JT, et al. International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia, for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). Blood 2006;108: 1497-503.
9. Jack FR, Smith SR, Saunders PW. Idiopathic myelofibrosis: anaemia may respond to low-dose dexamethasone. Br J Haematol 1994;87:877-8.

Increased cortical bone mineralization in imatinib treated patients with chronic myelogenous leukemia

Imatinib mesylate (Glivec®, Gleevec™, Novartis International AG) and the second generation ABL tyrosine kinase inhibitors have markedly improved the outcome of patients with chronic myeloid leukemia (CML). More patients are receiving treatment with these inhibitors for prolonged periods of time and experience with imatinib now exceeds five years. The immediate side effects are usually mild and manageable. There have been recent reports on the long-term side effects associated with prolonged use of imatinib.[1-3] Berman and coworkers found that imatinib treated patients had hypophosphatemia, lower osteocalcin levels and higher parathyroid hormone levels.[2] Subsequent studies confirmed the observation of hypophosphatemia in patients receiving imatinib.[4-8] The authors concluded that imatinib may affect bone remodeling and if left untreated, chronic hypophosphatemia may result in impaired bone mineralization, rickets, and osteomalacia.[2]

We, therefore, investigated bone mineral density (BMD) in imatinib treated CML patients and healthy controls. All imatinib treated CML patients at Sahlgrenska University Hospital were identified of whom 17 fulfilled the study inclusion criteria: (i) imatinib treatment duration ≥24 months, and (ii) first chronic phase of the disease with complete cytogenetic remission. The inclusion criteria were set to minimize the confounding effect of leukemia and to allow time for a pos-

Table 1. Blood counts, serum chemistry and bone related variables in imatinib treated chronic myeloid leukemia patients (n=17), and age and sex matched healthy controls (n=17). The results are given as mean ± SD.

	CML	Controls	p
Males/females	11/6	11/6	ns
Age (years)	60±11	59±11	ns
B-Hb, g/L	128±9	150±10	<0.001
B-Leukocyte count, $\times 10^9$/L	4.4±1.3	5.7±1.2	0.009
B-Platelet count, $\times 10^9$/L	221±79	257±50	ns
S-Sodium, mmol/L	141±2	141±3	ns
S-Potassium, mmol/L	4.1±0.3	4.2±0.2	0.024
S-Calcium, mmol/L	2.21±0.09	2.33±0.08	<0.001
S-Ionized Calcium, mmol/L	1.21±0.02	1.24±0.03	0.004
S-Phosphate, mmol/L	0.89±0.17	1.04±0.17	0.009
S-Magnesium, mmol/L	0.79±0.05	0.85±0.05	0.002
S-Creatinine, μmol/L	95±25	81±9	0.035
U-Calcium, mmol/L	2.14±1.07	3.35±1.97	0.034
tU-Calcium, mmol/24 hours	3.26±1.1	5.33±1.84	0.003
tU-Phosphate, mmol/24 hours	28±11	31±8	ns
S-Parathyroid hormone, ng/L	44±18	34±12	0.062
S-25-OH-vitamin D, μg/L	25.1±6.6	28.9±7.5	ns
S-1,25 (OH)2 vitamin D, ng/L	42.1±10.5	48.8±9.6	ns
P-Erythropoietin, IU/L	24.6±19.5	9.3±3.8	0.003
S-Osteocalcin, μg/L	6.8±2.0	10.1±3.9	0.004
S-Bone-specific alkaline phosphatase, μg/L	7.4±2	10.0±2.7	0.003
S-ICTP, μg/L	3.86±0.76	3.36±0.72	ns

ns: not significant.

Table 2. BMD measurements in imatinib treated chronic myeloid leukemia patients (n=17) and controls (n=17).

	DXA measurements	CML	Controls	p
Hip bone (total)	BMD, g/cm^2	1.08±0.2	0.95±0.1	0.025
	T-score	0.07±1.42	-0.82±0.83	
	Z-score	0.46±1.37	-0.26±0.85	
Lumbar spine (L1-4)	BMD, g/cm^2	1.27±0.22	1.12±0.15	0.029
	T-score	0.38±1.77	-0.82±1.23	
	Z-score	0.57±1.72	-0.36±1.29	
	pQCT-measurements			
Radius	Trabecular vBMD*, mg/cm^3	190.9±34·2	193.9±28.8	ns
	Cortical vBMD**, mg/cm^3	1211.3±23.8	1181.1±38.7	0.01
	Cortical area**, mm^2	95.1±16·3	88.6±18.7	ns
Tibia	Trabecular vBMD*, mg/cm^3	240.2±47.3	226.2±23.9	ns
	Cortical vBMD*, mg/cm^3	1185.6±23.5	1159.4±36.2	0.017
	Cortical area**, mm^2	262.6±50.7	261.3±44.4	ns

*The BMD was evaluated using two different techniques. The first was a two-dimensional measurement of the hip and lumbar spine bones using DXA. The second technique, pQCT, measures the volumetric BMD (vBMD) of the radius and tibia bones, and can separately analyze the cortical and trabecular bone compartments. DXA: the T-score is the number of standard deviations above or below the average for a young adult (20-40 years; same race and gender) at peak bone density. The reference material was from The Third National Health and Nutrition Examination Survey (NHANES III). The Z-score is the number of standard deviations above or below an average person of the same age, race and gender (reference material provided by GE Lunar Corp., Madison, WI). pQCT: vBMD and cross sectional area were measured at *4% and **25% bone length in the proximal direction. The results are given as mean ± SD.*

sible bone remodeling effect of imatinib to take place. Sex and aged matched healthy individuals served as controls (Table 1). The imatinib dose was 400 mg per day. The treatment duration was 50±19 months (range 24-73). The study was approved by the local research ethics committee. Details of design and methods are available as an online supplement.

The CML patients had significantly lower levels of calcium, ionized calcium, phosphate and magnesium in serum compared with controls (Table 1). Two out of 17 patients had serum ionized calcium below the reference range (1.18-1.31 mmol/L), 2 out of 17 patients had serum phosphate below the reference range (0.7-1.6 mmol/L) while none of the patients had serum magnesium below the reference range (0.7-0.95 mmol/L). The excretion of calcium in the urine was also found to be significantly lower in the imatinib treated patients (Table 1).The markers of bone turnover, osteocalcin and bone-specific alkaline phosphatase were significantly lower in patients compared with controls, whereas the marker of bone resorption carboxyterminal cross-linked telopeptide of type I collagen was unchanged (Table 1).

Our data confirm and extend those of Berman and coworkers despite differences in the patient and control design.[2] Berman *et al*. reported that imatinib treated CML and gastrointestinal stromal tumor patients with hypophosphatemia had a lower osteocalcin level, higher parathyroid hormone level and were treated with a higher imatinib dose (median 600 mg) than patients with a normal phosphate level. These findings were explained by an imatinib induced inhibition of bone turnover, which in turn, triggered a secondary hyperparathyroidism in an attempt to maintain calcium homeostasis. The authors raised concerns about an imatinib induced osteomalacia and suggested phosphate replacement therapy. In contrast to the hypothesized imatinib induced osteomalacia, we found that our imatinib treated CML patients had significantly higher areal BMD of the lumbar spine (+12%) and total hip bone (+12%) compared with controls (Table 2). Even compared with the standard reference, the patients had higher BMD than could be expected for age and gender. The patients' T-score value was 0.07 and 0.38 for the total hip bone and lumbar spine respectively (Table 2). The patients' Z-score value was 0.46 and 0.57 for total hip bone and lumbar spine (L1-4) respectively. A significant relationship was also observed between the imatinib treatment duration and areal BMD of the lumbar spine (L1-L4) (Spearman's ρ=0.49, p=0.046). Similarly, for both radius and tibia the imatinib treated CML patients had significantly higher cortical volumetric BMDs than the controls. No difference was seen in trabecular volumetric BMD of either radius or tibia. The cortical cross-sectional area was also similar in the two groups (Table 2).

Our results are also in line with previous *in vitro* studies and animal models. Osteoblasts are known to derive from mesenchymal stem cells, while osteoclasts are multinucleated giant cells of hematopoietic origin in the monocytic lineage. Both are targeted by imatinib and it has been shown that imatinib promotes osteoblast differentiation and inhibits osteoclastogenesis, presumably through its action on the colony-stimulating factor 1 receptor (C-FMS) and platelet derived growth factor receptor (PDGFR).[9,10]

Overall, biochemical findings suggest a suppressed bone turnover and the increased BMD indicates that bone formation and resorption are affected unequally. We suggest that imatinib uncouples bone formation from bone resorption in favor of the former, disturbing bone homeostasis and leading to a net increase in bone mineral density.

This is further supported by a recent study by Fitteri *et al.*,[11] who reported an increased trabecular bone volume in imatinib treated patients. In contrast to our study, they did not analyze the bone mineralization, but measured the trabecular bone volume on decalcified iliac crest biopsies obtained before and during imatinib treatment. Therefore, bone mineralization, the most important measure of osteopenia, was not addressed.

The present report is the first demonstrating altered calcium and phosphate metabolism in imatinib-treated CML patients, concomitant with an increased cortical bone mineralization. Our study alleviates previous concerns about an accelerated osteomalacia, and measures to prevent this hypothesized long-term side effect seem unnecessary and might even be harmful.[2,12]

If imatinib is shown to suppress bone resorption without decreasing the bone quality, tyrosine kinase inhibitors could be novel antiosteolytic agents in skeletal disorders.

Sofia Jönsson,[1] Bob Olsson,[1] Claes Ohlsson,[2] Mattias Lorentzon,[2] Dan Mellström,[2] and Hans Wadenvik[1]

[1]Haematology Section, Department of Internal Medicine, Gothenburg University, Gothenburg; [2]Center for Bone Research, Department of Internal Medicine, Gothenburg University, Gothenburg, Sweden

Funding: the study was supported by grants from the Swedish Research Council (project K2006-71X-11630-07B and project 529-2004-6512), FoU Västra Götaland, the Swedish Society for Medical Research, "JK-foundation" Sahlgrenska University Hospital, "Volvo Assar Gabrielssons foundation", the Foundations of the National Board of Health and Welfare, the Åke Wiberg Foundation, the Jeansson Foundation, the Tore Nilsson Foundation for Medical Research and the Magnus Bergvall Foundation.

Key words: imatinib, calcium, phosphate, chronic myelogenous leukemia, bone mineral density.

Correspondence: Sofia Jönsson, M.D., Haematology Section, Department of Internal Medicine, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden. Phone: international +46.31.3227782. Fax: international +46.31.416488. E-mail: sofia.jonsson@vgregion.se

Citation: Jönsson S, Olsson B, Ohlsson C, Lorentzon M, Mellström D, Wadenvik H. Increased cortical bone mineralization in imatinib treated patients with chronic myelogenous leukemia. Haematologica 2008; 93:1101-1103. doi: 10.3324/haematol.12373

References

1. Appel S, Balabanov S, Brummendorf TH, Brossart P. Effects of imatinib on normal hematopoiesis and immune activation. Stem Cells 2005;23:1082-8.
2. Berman E, Nicolaides M, Maki RG, Fleisher M, Chanel S, Scheu K, et al. Altered bone and mineral metabolism in patients receiving imatinib mesylate. N Engl J Med 2006; 354:2006-13.
3. Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 2006;12:908-16.
4. Grey A, O'Sullivan S, Reid IR, Browett P. Imatinib mesylate, increased bone formation, and secondary hyperparathyroidism. N Engl J Med 2006;355:2494-5.
5. Joensuu H, Reichardt P. Imatinib and altered bone and mineral metabolism. N Engl J Med 2006;355:628-9.
6. Osorio S, Noblejas AG, Duran A, Steegmann JL. Imatinib mesylate induces hypophosphatemia in patients with chronic myeloid leukemia in late chronic phase, and this effect is associated with response. Am J Hematol 2007;82:394-5.
7. Owen S, Hatfield A, Letvak L. Imatinib and altered bone and mineral metabolism. N Engl J Med 2006;355:627-9.
8. Zekri JM, Robinson MH, Woll PJ. Relative hypocalcaemia and muscle cramps in patients receiving imatinib for gastrointestinal stromal tumour. Sarcoma 2006;2006: 48948.
9. Dewar AL, Cambareri AC, Zannettino AC, Miller BL, Doherty KV, Hughes TP, et al. Macrophage colony-stimulating factor receptor c-fms is a novel target of imatinib. Blood 2005;105:3127-32.
10. O'Sullivan S, Naot D, Callon K, Porteous F, Horne A, Wattie D, et al. Imatinib promotes osteoblast differentiation by inhibiting PDGFR signaling and inhibits osteoclastogenesis by both direct and stromal cell-dependent mechanisms. J Bone Miner Res 2007;22:1679-89.
11. Fitter S, Dewar AL, Kostakis P, To LB, Hughes TP, Roberts MM, et al. Long term imatinib therapy promotes bone formation in CML patients. Blood 2008; 111:2538-47.
12. Schiffer CA. BCR-ABL tyrosine kinase inhibitors for chronic myelogenous leukemia. N Engl J Med 2007;357: 258-65.

Heterogeneous promoter activity of the telomerase reverse transcriptase gene in individual acute myeloid leukemia cells defined by lentiviral reporter assay

Acute myeloid leukemia (AML) generally constitutes a hierarchy of heterogeneous populations in terms of proliferation.[1,2] Currently, no phenotypic marker allows efficient discrimination of such cellular potential. One of the major intrinsic elements defining the replicative capacity of individual leukemia cells is telomerase activity.[3] Variable levels of telomerase activity have been detected in AML.[4,5] However, direct measurement of telomerase activity, such as the telomere repeat amplification protocol (TRAP) assay, detects only the mean value in a sample and is unable to isolate specific cell subpopulations. Ali et al. developed a method of detecting intranuclear hTERT protein using flow cytometry.[6] However, the sensitivity of this method appears low, and cell viability was lost by the procedure.

To dissect the heterogeneity of the proliferative potential in AML, a cell-based analysis of telomerase activity or related parameters is desired. Several investigators have shown that the telomerase activity in a variety of human tumors closely correlates with the mRNA level of its catalytic subunit, telomerase reverse transcriptase (hTERT),[7] which may be the best correlate of telomerase activity. Furthermore, the promoter activity of the hTERT gene was shown to correlate well with the hTERT mRNA expression as well as the telomerase activity.[8] Since plasmid-based reporter assays are not applicable to primary blood cells because of their extremely low transduction efficiency, lentivirus-mediated gene transfer is the most efficient approach in this respect.[9,10] We developed a cell-based assay for the promoter activity of the hTERT gene using a lentiviral vector and applied it to analyzing primary AML cells. A 1.2-kb upstream region of the hTERT gene was cloned from normal lymphocyte genomic DNA