Inhibitory effects of imatinib on vitamin D_3 synthesis in human keratinocytes

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Abstract. Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the presence of the *BCR-ABL1* fusion gene, a constitutively active, oncogenic tyrosine kinase that is responsible for the clinical features of CML. Tyrosine kinase inhibitors, such as imatinib, have markedly altered the treatment of CML. However, tyrosine kinase inhibitors are associated with side effects on bone metabolism, in adult and pediatric patients. Vitamin D_3 is involved in the complex cycle of bone remodeling, therefore the present study aimed to investigate the influence of imatinib on vitamin D_3 metabolism in the HaCaT human keratinocyte cell line, using commercially available enzyme assays. Imatinib was shown to significantly reduce the production of calcidiol and calcitriol. Based on interaction studies of imatinib with the cytochrome P450 (CYP450) inhibitors VID400 and ketoconazole, it is proposed that imatinib may interfere with the vitamin D_3 cascade due to its metabolism by CYP27B1, which is involved in vitamin D_3 metabolism.

Introduction

Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph^+) chromosome [t(9;22) q34;q11)] (1). This chromosome harbors the constitutively active, oncogenic tyrosine kinase (TK) Breakpoint Cluster Region-Abelson murine leukemia viral proto-oncogene 1 (BCR-ABL1), which is responsible for leukemic cell transformation (2-4). Imatinib mesylate (Glivec®/Gleevec®, Novartis, Basel, Switzerland) is a potent and selective inhibitor of BCR-ABL1. It was initially licensed in 2001 (5-10), and has since rapidly become the standard front-line treatment for CML, leading to high response rates (11). However, imatinib shows off-target effects on TKs other than BCR-ABL1, such as platelet-derived growth factor and colony-stimulating factor 1 receptor, which are involved in the bone remodeling cycle (12). Previous studies have revealed that prolonged imatinib treatment in adult CML patients may cause hypophosphatemia and altered bone mineralization (13-15), whereas pediatric CML patients develop growth retardation in ≤70% of cases (16,17).

Growth delay due to long-term imatinib intake is increasingly observed (11,12,16,18,19), and is more prominent in patients who began treatment with imatinib at prepubertal age (12). In addition, pediatric patients exhibit reduced serum levels of 25-hydroxyvitamin D_3 (25-OH-VD_3; calcidiol) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$-VD_3; calcitriol) (20) whilst on imatinib treatment. In humans, vitamin D_3 (VD_3) is synthesized by keratinocytes in the skin, by UVB-induced photolysis of 7-dehydrocholesterol (7-DHC), which results in the formation of previtamin D_3, followed by a thermal isomerization step (21). Thereafter, VD_3 is enzymatically hydroxylated to calcidiol by cytochrome P450 (CYP450) isoenzymes CYP2R1 and/or CYP27A1 (22) in the liver, and further metabolized to hormonally active calcitriol by CYP27B1 (23-25) in the kidney (Fig. 1). In order to investigate the calcitriol pathway and its modulation, the HaCaT human keratinocyte cell line was established by Lehmann (26) as a cellular model, thus demonstrating for the first time that HaCaT cells were capable of hydroxylating calcidiol to calcitriol.

Calcitriol is essential in regulating blood levels of calcium and phosphorus (27) and has a key role during bone mineralization (28-30). Numerous studies have identified an association of vitamin D_3 deficiency (as indicated by low calcidiol/calcitriol blood levels) with impaired growth, particularly during puberty and prepuberty (28,31). However, the detailed mechanisms causing growth delay during imatinib therapy are currently speculative. The aim of the present study was to investigate the effects of the TK inhibitor (TKI) imatinib on vitamin D_3 metabolism in the HaCaT human keratinocyte cell line.

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Materials and methods

Cell culture protocol. The HaCaT human keratinocyte cell line was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The cells were seeded at a density of $1x10^5$ cells/cm^2 and grown in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies GmbH, Darmstadt, Germany), supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies GmbH) in a 95% humidified atmosphere containing 5% CO_2, at 37˚C for 48 h. The media was subsequently replaced by serum-free DMEM for 18 h, in order to induce synchronization of the cell cycle. The cells were then grown in FBS-supplemented DMEM for 8 h, until they had reached 80-90% confluency. To investigate the metabolism of vitamin D_3, the cells were seeded at a density of $5x10^4$ cells/cm^2 in culture dishes (Ø, 30 mm).

Vitamin D_3 assay. To investigate vitamin D3 metabolism the HaCaT cells ($5x10^4$ cells/cm^2) were incubated with 25 μM 7-DHC (dissolved in 100% ethanol; Sigma-Aldrich, Steinheim, Germany) as a substrate, and exposed to UVB (300 nm; application rate, 75 mJ/cm^2). Irradiation of the cells was performed using a tuneable high intensity monochromator (FWHM, 5 nm; Müller Optik-Elektronik, Moosinning, Germany) over 15 min. At the start of irradiation the cells were incubated with imatinib (supplied by Novartis, Basel, Switzerland), at a concentration of 1 μM [dissolved in 100% dimethylsufloxide (DMSO)] for 24, 48, or 72 h. Following the incubation, the media and detached keratinocytes were collected and calcitriol was extracted using methanol : chloroform (1:1) (Merck, Darmstadt, Germany). The levels of calcitriol were determined quantitatively from the organic phase using a commercially available enzyme assay (1,25-Dihydroxy Vitamin D EIA; IDS, Frankfurt, Germany). All experiments were performed four times and the results were normalized to $1x10^6$ cells. Control experiments with ethanol and DMSO were conducted in order to identify any interactions with solvents or other components.

To determine whether the VD_3 processing CYP450 isoenzymes CYP2R1, CYP27A1 and CYP27B1 were inhibited by imatinib, specific inhibitors of the CYP450 isoenzyme family (VID400 and ketoconazole) were applied concomitantly. These experiments were conducted without irradiation. The HaCaT cells were incubated for 0, 2 or 4 h with either 5 μM cholecalciferol or 5 μM calcidiol (both dissolved in 100% ethanol) as a substrate. Prior to substrate incubation, the cells were incubated for 1 h with 200 nM VID400 or 10 μM ketoconazole (both dissolved in 100% ethanol), with or without 1 μM imatinib.

Statistical analysis. Statistical analysis at defined time points of incubation was performed using one-way analysis of variance with Bonferoni adjustment to evaluate the effects of IMA-treated samples compared with untreated controls, using the GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Inhibitory effects of imatinib on calcitriol synthesis. Imatinib incubation at the clinically effective concentration of 1 μM,

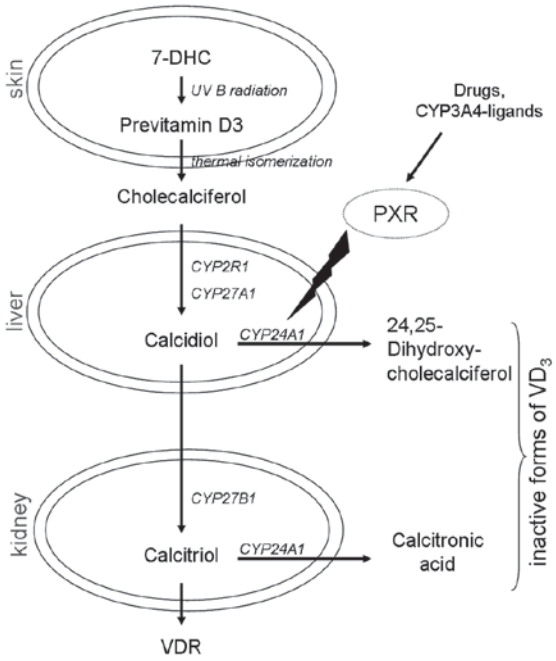

Figure 1. Vitamin D cascade and the enzymes involved. Modulation of CYP24A1 by citamin D metabolites or other compounds, such as PXR, may generate a high expression and vitamin D deficiency, as detected in various tumor tissues [modified after Schuster *et al* (32)]. VDR, vitamin D receptor; VD3, vitamin D_3; 7-DHC, 7-dehydrocholesterole; PXR, pregnane x receptor; CYP2R1, cytochrome P450 family 2, subfamily R, polypeptide 1 (vitamin D 25-hydroxylase); CYP27A1, cytochrome P450, family 27, subfamily A, polypeptide 1 (vitamin D 25-hydroxylase); CYP27B1, cytochrome P450, family 27, subfamily B, polypeptide 1 (1α-Hydroxylase); CYP24A1, cytochrome P450, family 22, subfamily a, polypeptide 1 (1,25-dihydroxyvitamin D_3 24-hydroxylase).

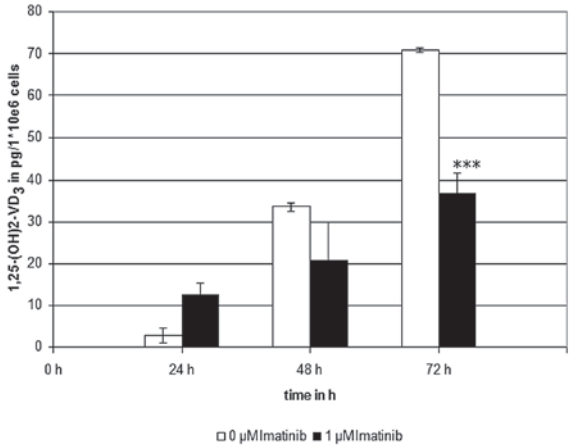

Figure 2. Time-dependent calcitriol ($1,25-(OH)_2-VD_3$) synthesis in the HaCaT human keratinocyte cell line following incubation with 7-dehydrocholesterole (7-DHC) as a substrate and UV irradiation (300 nm). The cells were either untreated (controls; white bars) or treated with imatinib (1 μM, black bars). The data represents the mean ± standard deviation of four experiments.

significantly reduced the calcitriol levels to ~50%, as compared with the controls, which were not treated with the TKI (Fig. 2). To verify these results, control experiments were conducted in the presence of 7-DHC without irradiation, and in the absence of 7-DHC with irradiation. Furthermore, to screen out any interactions of the solvents used, control experiments with

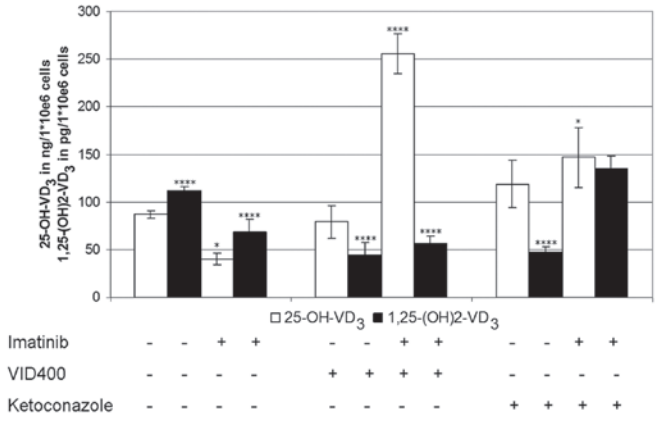

Figure 3. Synthesis of calcidiol (25-OH-VD3, white bars) and calcitriol (1,25-$(OH)_2$-VD3; black bars) in the HaCaT human keratinocyte cell line following incubation with cholecaliferol as a substrate. The cells were either untreated or treated with imatinib (1 μM) and inhibitors VID400 (200 nM) or ketoconazole (10 μM). The data are presented as the mean ± standard deviation of four experiments, in pg/1x10^6 cells for calcitriol and ng/1x10^6 cells for calcidiol.

ethanol and DMSO were conducted. As expected, no generation of calcitriol was detectable in the control experiments.

Effects of selective inhibitors in combination with imatinib on the vitamin D_3 cascade. Using cholecalciferol as the vitamin D_3 synthesis-starting substrate, the levels of calcidiol and calcitriol in the cells exposed to imatinib over 4 h were lowered to 50% that of the controls (Fig. 3). Treatment with the CYP450 inhibitors VID400 and/or ketoconazole, in the absence of imatinib, had nearly no effect on calcidiol levels (range, 90-110 ng/1x10^6 cells), whereas calcitriol levels decreased to 60% of the control values. Treatment with imatinib in the presence of VID400, resulted in increased calcidiol levels by 600% but had no effects on calcitriol synthesis. Treatment with ketoconazole and imatinib resulted in increased levels of calcitriol, by 200% (Fig. 3).

Furthermore, the experiments were repeated using calcidiol as the substrate and analyzed in the same way as previously described, resulting in calcitriol levels concordant with those described in Fig. 3, with the exception of ketoconazole. The cells incubated with imatinib in the absence of VID400 or ketoconazole had lower calcitriol levels, as compared with those incubated without imatinib. Identical levels were detected in the presence of VID400 without imatinib, whereas the combination of VID400 and imatinib increased the levels. In the presence of ketoconazole the calcitriol levels, with and without imatinib exposure, were decreased.

Discussion

During imatinib treatment longitudinal growth retardation has been identified as a frequent side effect in children (13-15,32-43). Jaeger *et al* (20) investigated biochemical skeletal markers in 17 pediatric patients with CML (age, 4-17 years) undergoing imatinib treatment and reported low serum levels of vitamin D_3, as well as impaired bone metabolism. However, children undergoing treatment for various types of cancer frequently exhibit vitamin D_3 deficiencies (44,45). The reason for this may be a lack of sun exposure or poor nutrition, but may also be due to drug interactions, or a combination of these factors (44).

In humans, vitamin D_3 has a primary role in maintaining extracellular ionized calcium levels and bone mineralization (46). In children, vitamin D_3 is required for growth and also for the prevention of rickets (47). In addition, vitamin D_3 is an important immunomodulator, that has been shown to have antiproliferative effects, potentiate apoptosis and inhibit angiogenesis (45). Pediatric oncology patients have a higher prevalence of vitamin D_3 hypovitaminosis (20,45). The present study aimed to investigate the reasons for vitamin D_3 deficiency and recognize the potentially causative mechanisms for low vitamin D serum levels and growth retardation in prepubertal patients with CML. The results of the present study demonstrate an inhibitory effect of imatinib on the synthesis of calcidiol and calcitriol during vitamin D_3 synthesis in human keratinocytes, leading to decreased levels by 50%. This finding is in concordance with the published clinical data of Jaeger *et al* (20).

To identify the potential target of imatinib within the vitamin D_3 cascade, the synthesis of calcidiol and calcitriol was examined in confluent HaCaT cells treated with two well-known specific CYP450 inhibitors: VID400 and ketoconazole. While ketoconazole is known to be a general inhibitor of P450 enzymes, VID400 specifically blocks CYP24A1, thus allowing the identification of the potential target of imatinib within the vitamin D_3 cascade with enzymes involved, such as CYP24A1, CYP27A1 and CYP27B1 (Fig. 1). Experiments were conducted in combination with and without imatinib using cholecalciferol as a substrate, therefore no irradiation of the cells was required. As previously described, VID400 at a concentration of 200 nM, may dose-dependently inhibit CYP24A1 activity, and partially inhibit CYP27B1 by 30% (48). Ketoconazole, at a concentration of 10 μM, is a general inhibitor of the CYP450 isoenzymes (49), including vitamin D hydroxylating enzymes, such as CYP24A1, CYP27A1 and CYP27B1 (50). The present study demonstrated that exposure to VID400 alone stabilized the levels of endogenously produced calcitriol.

It has been shown that VID400 results in increased expression of Cyp24 (451,52). CYP24 catalyzes the metabolism of calcidiol and calcitriol. The activity is regulated by a negative feedback loop dependent on calcitriol concentration, resulting in decreased calcitriol levels. It has previously been suggested, that in cancer cells, particularly in prostate cancer, a rapid breakdown of calcitriol levels is caused by an overactive CYP24 (53). The combination of the CYP24 inhibitors tested with imatinib, resulted in increased levels of calcidiol levels. These results suggest that besides the inhibition of CYP24 by VID400, the activity of CYP27B1 is impaired by imatinib, resulting in an intracellular accumulation of calcidiol. This is in concordance with a previous *in vivo* study, where it was shown that imatinib is metabolized by various liver CYP450 isoenzymes, mainly CYP3A4 and CYP3A5 (54). CYP3A4 is also known to be a human microsomal vitamin D 25-hydroxylase (55), similar to CYP27B1.

Isolated ketoconazole exposure resulted in increased calcidiol and decreased calcitriol levels, whereas the combination with imatinib increased the levels of calcidiol and calcitriol. Ketoconazole is also known to be a strong inhibitor

of CYP3A4 (56), resulting in poor metabolism of imatinib. Based on a drug interaction study, co-administration of imatinib and inhibitors as well as inducers of CYP3A4 activity (57), requires careful monitoring of the patients to rule out toxic side effects, or decreased TKI effects on the underlying CML.

To catalyze the 25-hydroxylation step in the liver, at least six CYPs are involved *in vivo*, the most prominent ones being CYP27A1 and CYP2R1 (58). CYP27B1 is responsible for the renal 1α-hydroxylation of vitamin D to hormonally active calcitriol. The vitamin D synthesis cascade is a complex system with numerous enzymes involved at diverse steps; therefore, various enzymes may be affected by imatinib. Imatinib, the inhibitors (VID400 and/or ketoconazole) and the substrates (cholecalciferol and/or calcidiol) may all compete for binding to one or more CYPs in keratinocytes, resulting in interference with vitamin D_3 metabolism. The results of the present study clearly indicate a competitive inhibition of CYP27B1 by imatinib, as concomitant blocking of CYP27B1 with VID400 resulted in elevated levels of calcidiol, but decreased levels of calcitriol. However, the mechanism remains poorly understood, and additional studies are required.

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References

1. Tipping AJ, Mahon FX, Zafirides G, *et al*: Drug responses of imatinib mesylate-resistant cells: synergism of imatinib with other chemotherapeutic drugs. Leukemia 16: 2349-2357, 2002.
2. Capdeville R, Silberman S and Dimitrijevic S: Imatinib: the first 3 years. Eur J Cancer 38: S77-S82, 2002.
3. Daley GQ, Van Etten RA and Baltimore D: Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247: 824-830, 1990.
4. Cohen MH, Williams G, Johnson JR, *et al*: Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 8: 935-942, 2002.
5. Champagne MA, Capdeville R, Krailo M, *et al*: Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. Blood 104: 2655-2660, 2004.
6. Druker BJ, Tamura S, Buchdunger E, *et al*: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2: 561-566, 1996.
7. Druker BJ, Talpaz M, Resta DJ, *et al*: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344: 1031-1037, 2001.
8. Grigg A and Hughes T: Role of allogeneic stem cell transplantation for adult chronic myeloid leukemia in the imatinib era. Biol Blood Marrow Transplant 12: 795-807, 2006.
9. Millot F, Guilhot J, Nelken B, *et al*: Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. Leukemia 20: 187-192, 2006.
10. Roy L, Guilhot J, Krahnke T, *et al*: Survival advantage from imatinib compared with the combination interferon-alpha plus cytarabine in chronic-phase chronic myelogenous leukemia: historical comparison between two phase 3 trials. Blood 108: 1478-1484, 2006.
11. Hobernicht SL, Schweiger B, Zeitler P, Wang M and Hunger SP: Acquired growth hormone deficiency in a girl with chronic myelogenous leukemia treated with tyrosine kinase inhibitor therapy. Pediatr Blood Cancer 56: 671-673, 2011.
12. Shima H, Tokuyama M, Tanizawa A, *et al*: Distinct impact of imatinib on growth at prepubertal and pubertal ages of children with chronic myeloid leukemia. J Pediatr 159: 676-681, 2011.
13. Berman E, Nicolaides M, Maki RG, *et al*: Altered bone and mineral metabolism in patients receiving imatinib mesylate. N Engl J Med 354: 2006-2013, 2006.
14. Fierro F, Illmer T, Jing D, *et al*: Inhibition of platelet-derived growth factor receptorbeta by imatinib mesylate suppresses proliferation and alters differentiation of human mesenchymal stem cells *in vitro*. Cell Prolif 40: 355-366, 2007.
15. Fitter S, Dewar AL, Kostakis P, *et al*: Long-term imatinib therapy promotes bone formation in CML patients. Blood 111: 2538-2547, 2008.
16. Schmid H, Jaeger BA, Lohse J and Suttorp M: Longitudinal growth retardation in a prepuberal girl with chronic myeloid leukemia on long-term treatment with imatinib. Haematologica 94: 1177-1179, 2009.
17. Suttorp M, Yaniv I and Schultz KR: Controversies in the treatment of CML in children and adolescents: TKIs versus BMT? Biol Blood Marrow Transplant 17: S115-S122, 2011.
18. Kimoto T, Inoue M and Kawa K: Growth deceleration in a girl treated with imatinib. Int J Hematol 89: 251-252, 2009.
19. Mariani S, Giona F, Basciani S, Brama M and Gnessi L: Low bone density and decreased inhibin-B/FSH ratio in a boy treated with imatinib during puberty. Lancet 372: 111-112, 2008.
20. Jaeger BA, Tauer JT, Ulmer A, *et al*: Changes in bone metabolic parameters in children with chronic myeloid leukemia on imatinib treatment. Med Sci Monit 18: CR721-CR728, 2012.
21. Lehmann B, Sauter W, Knuschke P, Dressler S and Meurer M: Demonstration of UVB-induced synthesis of 1 alpha, 25-dihydroxyvitamin D3 (calcitriol) in human skin by microdialysis. Arch Dermatol Res 295: 24-28, 2003.
22. Lehmann B and Meurer M: Vitamin D metabolism. Dermatol Ther 23: 2-12, 2010.
23. Holick MF: Vitamin D deficiency. N Engl J Med 357: 266-281, 2007.
24. Holick MF: Resurrection of vitamin D deficiency and rickets. J Clin Invest 116: 2062-2072, 2006.
25. DeLuca HF: Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 80: 1689S-1696S, 2004.
26. Lehmann B: HaCaT cell line as a model system for vitamin D3 metabolism in human skin. J Invest Dermatol 108: 78-82, 1997.
27. Bogh MK, Schmedes AV, Philipsen PA, Thieden E and Wulf HC: Interdependence between body surface area and ultraviolet B dose in vitamin D production: a randomized controlled trial. Br J Dermatol 164: 163-169, 2011.
28. Kremer R, Campbell PP, Reinhardt T and Gilsanz V: Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. J Clin Endocrinol Metab 94: 67-73, 2009.
29. Davis CD and Dwyer JT: The 'sunshine vitamin': benefits beyond bone? J Natl Cancer Inst 99: 1563-1565, 2007.
30. Mathieu C and Badenhoop K: Vitamin D and type 1 diabetes mellitus: state of the art. Trends Endocrinol Metab 16: 261-266, 2005.
31. Pettifor JM: Rickets and vitamin D deficiency in children and adolescents. Endocrinol Metab Clin North Am 34: 537-553, 2005.
32. Schuster I, Egger H, Herzig G, Reddy GS, Schmidt JA, Schüssler M and Vorisek G: Selective inhibitors of vitamin D metabolism - new concepts and perspectives. Anticancer Res 26: 2653-2668, 2006.
33. Tibullo D, Giallongo C, La Cava P, *et al*: Effects of imatinib mesylate in osteoblastogenesis. Exp Hematol 37: 461-468, 2009.
34. O'Sullivan S, Naot D, Callon K, *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 22: 1679-1689, 2007.
35. Dewar AL, Zannettino AC, Hughes TP and Lyons AB: Inhibition of c-fms by imatinib: expanding the spectrum of treatment. Cell Cycle 4: 851-853, 2005.

36. Dewar AL, Cambareri AC, Zannettino AC, *et al*: Macrophage colony-stimulating factor receptor c-fms is a novel target of imatinib. Blood 105: 3127-3132, 2005.
37. Dewar AL, Domaschenz RM, Doherty KV, Hughes TP and Lyons AB: Imatinib inhibits the *in vitro* development of the monocyte/macrophage lineage from normal human bone marrow progenitors. Leukemia 17: 1713-1721, 2003.
38. Owen S, Hatfield A and Letvak L: Imatinib and altered bone and mineral metabolism. N Engl J Med 355: 627-629, 2006.
39. O'Sullivan S, Horne A, Wattie D, *et al*: Decreased bone turnover despite persistent secondary hyperparathyroidism during prolonged treatment with imatinib. J Clin Endocrinol Metab 94: 1131-1136, 2009.
40. El Hajj Dib I, Gallet M, Mentaverri R, *et al*: Imatinib mesylate (Gleevec) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. Eur J Pharmacol 551: 27-33, 2006.
41. Grey A, O'Sullivan S, Reid IR and Browett P: Imatinib mesylate, increased bone formation, and secondary hyperparathyroidism. N Engl J Med 355: 2494-2495, 2006.
42. Jönsson S, Olsson B, Ohlsson C, *et al*: Increased cortical bone mineralization in imatinib treated patients with chronic myelogenous leukemia. Haematologica 93: 1101-1103, 2008.
43. Vandyke K, Fitter S, Dewar AL, Hughes TP and Zannettino AC: Dysregulation of bone remodeling by imatinib mesylate. Blood 115: 766-774, 2010.
44. Genc DB, Ozkan MA and Buyukgebiz A: Vitamin D in childhood cancer: a promising anticancer agent? Pediatr Endocrinol Rev 10: 485-493, 2013.
45. Helou M, Ning Y, Yang S, *et al*: Vitamin D deficiency in children with cancer. J Pediatr Hematol Oncol 36: 212-217, 2014.
46. Mithal A, Wahl DA, Bonjour JP, *et al*: Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 20: 1807-1820, 2009.
47. Lips P: Vitamin D status and nutrition in Europe and Asia. J Steroid Biochem Mol Biol 103: 620-625, 2007.
48. Xie Z, Munson SJ, Huang N, *et al*: The mechanism of 1,25-dihydroxyvitamin D(3) autoregulation in keratinocytes. J Biol Chem 277: 36987-36990, 2002.
49. Nguyen M, Boutignon H, Mallet E, *et al*: Infantile hypercalcemia and hypercalciuria: new insights into a vitamin D-dependent mechanism and response to ketoconazole treatment. J Pediatr 157: 296-302, 2010.
50. Segersten U, Björklund P, Hellman P, Akerström G and Westin G: Potentiating effects of nonactive/active vitamin D analogues and ketoconazole in parathyroid cells. Clin Endocrinol (Oxf) 66: 399-404, 2007.
51. Schuster I, Egger H, Reddy GS and Vorisek G: Combination of vitamin D metabolites with selective inhibitors of vitamin D metabolism. Recent Results Cancer Res 164: 169-188, 2003.
52. Schuster I, Egger H, Nussbaumer P and Kroemer RT: Inhibitors of vitamin D hydroxylases: structure-activity relationships. J Cell Biochem 88: 372-380, 2003.
53. Yee SW, Campbell MJ and Simons C: Inhibition of Vitamin D3 metabolism enhances VDR signalling in androgen-independent prostate cancer cells. J Steroid Biochem Mol Biol 98: 228-235, 2006.
54. Peng B, Lloyd P and Schran H: Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 44: 879-894, 2005.
55. Gupta RP, Hollis BW, Patel SB, Patrick KS and Bell NH: CYP3A4 is a human microsomal vitamin D 25-hydroxylase. J Bone Miner Res 19: 680-688, 2004.
56. Takeshita A, Taguchi M, Koibuchi N and Ozawa Y: Putative role of the orphan nuclear receptor SXR (steroid and xenobiotic receptor) in the mechanism of CYP3A4 inhibition by xenobiotics. J Biol Chem 277: 32453-32458, 2002.
57. Dagher R, Cohen M, Williams G, *et al*: Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res 8: 3034-3038, 2002.
58. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ and Russell DW: Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101: 7711-7715, 2004.