Multiparameter Analysis of Off-Target Effects of Dasatinib on Bone Homeostasis in Patients With Newly Diagnosed Chronic Myelogenous Leukemia

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Abstract

We assessed patients with chronic myelogenous leukemia for serum Ca, PO_4, bone alkaline phosphatase, N-telopeptide, osteoprotegerin levels and trabecular bone (TBA) in bone marrow (BM) specimens before and after treatment with dasatinib. We identified a significant increase in TBA % in post-dasatinib BM ($P = .022$). This suggests that dasatinib therapy can increase TBA, without significant changes in bone and mineral metabolism.

Background: We assessed patients with chronic myelogenous leukemia (CML) for serum calcium (Ca), phosphate (PO_4), bone alkaline phosphatase, N-telopeptide (NTx), osteoprotegerin (OPG) levels, and trabecular bone area (TBA) in bone marrow (BM) specimens before and after treatment with dasatinib. We identified a significant increase in percentage of TBA in postdasatinib BM ($P = .022$). This suggests that dasatinib therapy can increase TBA without significant changes in bone and mineral metabolism. Interferences with bone homeostasis and mineral metabolism have been described in patients receiving imatinib for CML or gastrointestinal stromal tumors. Dasatinib is a potent second-generation tyrosine kinase inhibitor designed to inhibit *ABL* and SRC kinases while also interfering with the c-Kit, platelet-derived growth factor receptor, and STAT5 pathways. **Patients and Methods:** We used a multiparameter approach to examine the off-target effects of dasatinib in 30 patients with CML treated between 2009 and 2012. We recorded serum Ca and PO_4 levels, analyzed markers of bone formation (bone alkaline phosphatase/bone-specific alkaline phosphatase [BAP]) and bone resorption (NTx), measured OPG levels, and digitally analyzed changes in TBA in paired BM biopsy specimens before and after treatment. We correlated all findings with each other and with the results of conventional cytogenetic and molecular analyses. **Results:** We identified a significant increase in the percentage of TBA in postdasatinib BM biopsy specimens ($P = .022$) and noted a decrease in serum OPG levels in 75% of patients. Ca, PO_4, BAP, and NTx levels remained steady, without significant changes. There was no correlation between biomarker levels, percentage of TBA, and/or cytogenetic or molecular response. **Conclusion:** These findings suggest that dasatinib therapy (within the therapeutic range) can increase trabecular bone, without causing significant changes in bone and mineral metabolism. Nonetheless, monitoring of bone health and skeletal integrity should be included into the long-term management of patients treated with dasatinib to further enhance our understanding of its safety profile and its potential role as a treatment modality for other bone diseases.

Clinical Lymphoma, Myeloma & Leukemia, Vol. 16, No. S1, S86-92
Keywords: Bone marrow, Bone metabolism, Dasatinib, Digital image analysis, Trabecular bone

Presented in part as abstract 1682 at the American Society of Hematology 2012 annual meeting.

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Submitted: Feb 9, 2016; Accepted: Feb 9, 2016

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2152-2650/$ - see frontmatter
http://dx.doi.org/10.1016/j.clml.2016.02.027

Introduction

With the introduction of targeted oral tyrosine kinase inhibitor (TKI) therapy, long-term or lifelong therapy for patients with chronic myelogenous leukemia (CML) is now a reality. Imatinib (Novartis, Basel, Switzerland) was the first TKI approved for the treatment of CML and was considered the standard of care for more than a decade. Notably, many in vitro studies demonstrated that imatinib can interfere with bone hemostasis by affecting the morphology and function of osteoclasts and osteoblasts through the c-fms (Susan McDonough strain of feline sarcoma virus), c-abl (Abelson murine leukemia viral oncogene homolog 1), and PDGF (platelet-derived growth factor) receptor pathways. These result in alterations of the OPG/RANK/RANKL (osteoprotegerin/receptor activator of nuclear factor κ/receptor activator of nuclear factor kappa beta ligand) system, thus increasing the expression of osteogenic markers, such as osteocalcin, RUNX2 (Runt-related transcription factor 2), and BMP2 (bone morphogenetic protein 2).[1-6] Simultaneously, several in vivo studies corroborated these findings, noting that CML patients undergoing treatment with first-generation TKIs had indicators of altered bone metabolism in their peripheral blood as well as changes in bone mineral density as identified on serial dual energy x-ray absorptiometry scans shortly after commencing therapy.[7-10]

Dasatinib (Bristol-Myers Squibb) is a multitargeted, second-generation kinase inhibitor that was initially used in patients with disease resistant or intolerant to imatinib front-line therapy.[11-14] Originally the drug was developed as an inhibitor of Abl and SRC family kinases (Rous sarcoma virus oncogene protein pp60 [V-SRC]) including c-Src, Lck, Hck, Yes, Fgr, Lyn, and Fyn.[11] Dasatinib also inhibits PDGF family members, particularly c-Kit and PDGF receptor α/β, at therapeutic levels.[15] In addition, it can bind to MAPK (mitogen-activated protein kinases), a tyrosine kinase discoidin domain receptor, and can block downstream signaling of STAT5 (signal transducer and activator of transcription 5), which down-regulates expression of STAT5 target genes, including *Bcl-x, Mcl-1,* and *cyclin D1*.[16] Dasatinib has an enhanced affinity for the *BCR-ABL1* fusion transcript, can block *BCR-ABL1* phosphorylation, and is less sensitive to the common mutations in the *ABL* kinase domain.[14,17] Thus, the drug is more potent through its multitargeted profile. Dasatinib resulted in significantly higher and faster rates of complete cytogenetic and major molecular responses (MMR) in CML patients compared to imatinib and is approved by the US Food and Drug Administration as a first-line therapy.[18-21]

Considering the effects of the first-generation TKIs on bone homeostasis, it is no surprise that many in vitro studies were able to demonstrate that the second-generation reagent, with its higher potency and its broader effect on different kinase families, can also influence bone metabolism.[22-26] Dasatinib can disturb the balance between osteoblast–osteoclast interactions by producing a convergent effect comprising increased bone anabolism and reduced bone resorption.[27] Interestingly, these results subsequently led several authors to propose a potential (off-target, off-label) application for dasatinib in the treatment of other bone-specific disorders,[28-30] which mandates the necessity of conducting comprehensive studies analyzing bone health in long-term users.

Our goal in this study was to assess the off-target effects of dasatinib monotherapy on bone homeostasis in chronic phase CML patients in order to better understand its effects on bone health in patients in general. We recorded calcium (Ca) and PO_4 levels and measured levels of 4 additional selected biomarkers of bone health, namely serum and urinary N-telopeptide (NTx) of collagen type 1, a marker of bone resorption; serum alkaline phosphatase isoenzyme/bone-specific alkaline phosphatase (BAP), a marker of osteoblast activity and bone matrix calcification; and serum OPG, an osteoclastogenesis inhibitory factor established to have bone-protective effects through the OPG/RANK/RANKL system. We also quantified trabecular bone areas (TBA) in bone marrow (BM) biopsy specimens via digital image analysis, as described previously,[31] tabulated cytogenetic and molecular treatment responses, and correlated all findings with each other.

Patients and Methods

Patient Selection

We prospectively identified adult patients with newly diagnosed Philadelphia chromosome (Ph)-positive chronic phase CML who were being enrolled onto a preexisting clinical trial that was approved by the relevant institutional review boards and ethics committees. All patients provided informed written consent in accordance with the Declaration of Helsinki. Inclusion criteria encompassed adequate organ function and no other serious medical conditions. Each patient was scheduled to undergo periodic BM aspiration and biopsy, cytogenetic and molecular analyses, and serum and urine biomarker testing as part of the protocol. The first BM examination was performed at the time of study enrollment; a second BM specimen was obtained after 6 months of therapy; and a third BM specimen was obtained 1 year after commencing dasatinib monotherapy.

Cytogenetic and Molecular Analysis

Conventional cytogenetic analysis and fluorescence in-situ hybridization were performed on metaphase cells prepared from BM aspirate smears using standard techniques. Cytogenetic responses were classified according to degree of decreased in the Ph^+ metaphases compared to the pretreatment value. The categories included: no cytogenetic response (continued presence of 100% Ph^+ metaphases), minor cytogenetic response (35%-90% Ph^+ metaphases), partial cytogenetic response (5%-34% Ph^+ metaphases), and complete cytogenetic response (CCyR) with 0% Ph^+ metaphases.

A quantitative real-time reverse transcriptase polymerase chain reaction assay was used for assessment of *BCR-ABL1* transcript levels as described previously.[32] The assay was designed to detect residual leukemia with up to 4- to 5-log reduction from the baseline levels at diagnosis. A MMR was defined as a *BCR-ABL1/ABL1* ratio of $< 0.1\%$, and complete molecular response was defined as undetectable *BCR-ABL1* levels. Missing samples were counted as no response.

Digital Image Analysis of BM Biopsy Specimens

We used a digital image analysis algorithm to examine BM specimens using a method similar to one described previously.[31] Limited-quality or suboptimal-quality biopsy specimens (substantial crush or aspiration artifact) and subcortical biopsy specimens were excluded from analysis. Routine hematoxylin and eosin–stained slides from formalin-fixed, paraffin-embedded BM biopsy samples were digitally