INTRODUCTION

Despite recent advances in therapy, multiple myeloma (MM) remains an incurable disease. MM-associated osteolytic bone disease (OBD), which occurs in more than 80% of MM patients, is a major cause of morbidity and mortality in MM and the development of new therapeutic strategies is of great interest. Underlying MM-associated OBD is an uncoupling of the bone remodeling process, with an increased activity of osteoclasts (OCL) and a decreased activity of osteoblasts (OBL). SRK kinase has been implicated in both OCL and OBL function. In this study, we assessed the effect of saracatinib (AZD0530, AstraZeneca) on the development of MM and its associated OBD.

MATERIALS AND METHODS

Saracatinib. Saracatinib was kindly provided by AstraZeneca. For in vitro studies a stock solution was prepared in DMSO. For in vivo studies, saracatinib was dissolved in 0.5% hydroxy-propyl-methyl-cellulose containing 0.1% Tween-80. Mice received a daily dose of 25mg/kg saracatinib or vehicle by oral gavage.

OCL assays. Murine bone marrow monocyte-derived OCLs (mOCLs) were generated in differentiation medium supplemented with 100ng/ml M-CSF and 100ng/ml sRANKL. RAW264.7 cultures: 30ng/ml sRANKL. TRAP activity was assessed with the Leukocyte TRAP kit (Sigma-Aldrich). Resorption was assessed on Osteo Assay plates (Corning) with a Von Kossa staining.

OBL assays. Murine mesenchymal stem cell (mMSC) and MC3T3-E1-derived OBLs were generated in differentiation medium supplemented with 50mg/ml ascorbic acid and 2mM β-glycerolphosphate. Von Kossa staining was performed to detect mineralized matrix and Sirius red staining to detect collagen.

Murine MM models. 2.5 x 10^5 STGJM16GFP+ cells were injected i.v. in C57BL/Kalvar mice. 30 days post-injection, the mice were sacrificed and bone marrow plasmoscytoma was determined by FACS. The STGMM model is propagated by the transfer of 2 x 10^5 cells from diseased mice in younger syngeneic C57BL/Kalvar mice. 10 days post-transfer, the mice were sacrificed and bone marrow plasmoscytoma was determined by staining cytospins with May-Grünwald-Giemsa. In both models, spleens were weighed at sacrifice.

μCT. μCT was performed on a SkyScan1172 scanner (Bruker) with the following settings: 50Kv/200uA, 0.5mm aluminum filter, 5 mm² pixel size, 0.4° rotation. Morphometric analyses of trabecular and cortical bone was performed on proximal tibia and distal femur respectively.

Immunohistomorphometry. TRAP (OCL) and Toluidine blue (OBL) stainings were performed on methyl-methacrylate-embedded bone sections.

RESULTS

1. Saracatinib inhibits osteoclast differentiation and function in vitro

2. Saracatinib inhibits collagen secretion by osteoblasts and alters matrix mineralization in vitro

3. Saracatinib limits the development of osteolytic bone disease in multiple myeloma

CONCLUSIONS

This study further establishes SRC inhibition as a promising approach for the treatment of MM-associated OBD. We show a potent inhibitory effect of the SRC kinase inhibitor saracatinib on the development of OBD in both a prolymphocytic (STGJM1) and therapeutic (ST2MM) setting. Similar to the OCL phenotype in Src/- mice (Boyce et al., 1992), this likely occurs through an impairment of bone resorption.

Our study warrants the evaluation of bone parameters in MM patients treated with saracatinib or with novel similar compounds such as AZD0530 (clinicaltrials.gov: NCT01668550).

Figure 1. (A)-(H) Concentration-dependent inhibition of primary mOCL differentiation following saracatinib (S) treatment (TRAP staining). (C)(D) Concentration-dependent inhibition of mOCL matrix resorption following saracatinib treatment (Von Kossa staining). (E) mRNA expression of OCL markers following saracatinib treatment (RAW264.7 cells, qPCR).

Figure 2. (A)+(B) Saracatinib (S) inhibits MC3T3-E1- and primary mMSC-derived OBL collagen secretion (Sirius red staining). (A)+(B) Saracatinib alters MC3T3-E1- and primary MSC-derived OBL matrix mineralization (Von Kossa staining). (C) mRNA expression of OBL markers (MC3T3-E1 cells, qPCR).

Figure 3. (A)+(B) Saracatinib (S) reduces the number of cortical perforations in STGJM1 mice compared to vehicle (V)-treated mice. Perforations were counted manually on radiographs. (C) Saracatinib restores trabecular bone parameters in STGJM1 mice, including (D) trabecular bone volume (Tb. BV/TV), (E) trabecular number (Tb.N) and (F) trabecular thickness (Tb.Th). (G) Trabecular separation (Tb.Sp). (H)+(I) Saracatinib partially restores cortical thickness (Ct.Th) in STGJM1 mice. (J)+(K) Saracatinib does not reduce the number of cortical perforations in ST2MM mice. (L) Saracatinib restores trabecular bone parameters in ST2MM mice, including (M) trabecular bone volume (Tb. BV/TV), (N) trabecular number (Tb.N) and (P) trabecular separation (Tb.Sp). (Q) Trabecular thickness (Tb.Th). (R)+(S) Saracatinib does not restore cortical thickness (Ct.Th) in ST2MM mice.

Figure 5. (A) Saracatinib (S) does not restore (B) osteoid surface (OS/BS) or (C) OBL surface (Ob.S/BS) in STGJM1 mice (Aniline blue staining). Saracatinib results in a trend towards a decrease in (D) OCL surface (Oc.S/BS) and (E) OCL number (Oc.N) in STGJM1 mice (TRAP staining).