Reviewer’s report

Title: The Src inhibitor dasatinib stimulates the differentiation of human bone marrow-derived mesenchymal stromal cells into osteoblasts

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Reviewer: Susannah M O’Sullivan

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General Comments:
This a well presented and focused study on the effects of dasatinib to stimulate differentiation of human bone marrow-derived mesenchymal stromal cells (MSC) into osteoblasts, and inhibit expression of RANKL, thus potentially indirectly effecting osteoclast maturation and activation.

This study is interesting in light of the known effects of imatinib on osteoblast differentiation and osteoclast activation, and the effects of Src inhibition on osteoblast function in vivo and in vitro.

The authors have appropriately ensured the reliability of the differentiation assay, established appropriate gene and functional markers of osteoblast maturation, and the appropriate dose range of dasatinib in their assays. A number of different functional and gene markers have been used. The observations are robust and of significant scientific interest. However the authors have not addressed the possibility that the effects they have observed are due to inhibition of Abl or another tyrosine kinase. Furthermore, the limitations of the study are not clearly identified.

Specific Points:

Discretionary Revisions:
In the Background, the second sentence is somewhat cumbersome and could be revised. In the last sentence of this section, ‘osteogenic differentiation’ would be more appropriately termed ‘osteoblastic differentiation’.

Why have the authors chosen to use RT-PCR rather than real-time PCR (QT-PCR) in assessing relative gene expression?

Minor Essential Revisions:
In the Abstract, Brc-Abl should read Bcr-Abl. The first sentence of Methods subsection does not make sense and should be revised.

The last paragraph in the Results subsection ‘determination of osteoblast-related gene expression during MSC differentiation’ largely reduplicates information in the third paragraph in this section. These two paragraphs should be amalgamated.

In the Discussion paragraph 6, the authors comment that their data ‘strongly
support an addition indication for dasatinib therapy in patients with bone loss’. This is overstating the data; however it would be reasonable to assess the effects of dasatinib in an in vivo model.

Major Compulsory Revisions:

In the Background, some discussion of the relative inhibition of Bcr-Abl vs Src, as well as the known effects on bone of Abl inhibition should be incorporated. Are any other tyrosine kinases effected by dasatinib? What steps have been taken to ensure that the observed effects are (purely) due to Src inhibition? In the Discussion, some further justification for attributing the observed effects to Src should be included, particularly given the similar effects on osteoblast differentiation and RANKL expression to those seen with imatinib, which is a PDGF/Kit/Abl/c-Fms inhibitor. While the observed effects are robust, the attribution to Src-inhibition is largely based upon circumstantial evidence, and this limitation should be acknowledged. If the authors’ own work with another specific Src inhibitor is to be used as evidence that the effects are due to Src inhibition, these data should be presented.

As acknowledged by the authors, the functional and gene expression results from the differentiation experiments are not completely consistent, in particular the difference between ALP activity and ALP gene expression. Some further discussion should be provided regarding the reason for the discrepancies, and the reasons for the authors selecting certain markers as being more reliable than others. Furthermore, some attempt should be made at explaining dasatinib’s varying effect on gene expression with or without ‘DAG’.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare I have no competing interests.