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: Dorit Naot

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The study investigates the effects of dasatinib on osteoblast differentiation. Examining the activity of dasatinib in bone is interesting and important and the biological question of the study is well-defined. The methods used in the study are described in detail, and the challenging experimental system of in vitro differentiation of human osteoblasts and formation of mineralised bone nodules was used with reproducible results.

Major Compulsory Revisions

1. The authors use RT-PCR to measure changes in levels of gene expression. The PCR parameters detailed in the Methods section indicate 35 cycles of amplification in each reaction. Under these conditions the PCR is not quantitative and cannot be described as ‘semi-quantitative’. The major concern here is that the house keeping gene #-actin, which is usually highly expressed, has reached saturation levels and therefore could not be used to normalise the levels of expression of the other target genes. The exponential phase of the PCR amplification for each of the probe sets should be determined and the experiments should be repeated with the appropriate, empirically determined number of cycles for each gene.

Discretionary Revisions

1. The great majority of the work focuses on the development and evaluation of the experimental system while dasatinib effects are only shown in figures 7 and 8. As figure 8 presents a gene expression study it requires revision. The manuscript could be improved by the addition of a bone nodule assay, similar to the one presented in figure 2A, for the study of the effect of dasatinib on nodule formation.

2. The decrease in expression of RANKL is interpreted as a possible indirect mechanism of inhibition of osteoclastogenesis by dasatinib. However, if the RT-PCR results are correct, the decrease in RANKL only occurs in the absence of DAG, while in the presence of DAG there seems to be a slight increase in RANKL expression. Also, the levels of OPG should have been determined in this experiment, as the ratio between RANKL /OPG is a more accurate indicator of the effect of osteoblasts on osteoclastogenesis.

3. Table 2 presents the same data as figure 4 and could be omitted.
Minor Essential Revision
1. ‘Brc-Abl’ appears in a number of places and should be corrected to ‘Bcr-Abl’.
2. The last sentence of the introduction is unclear (...in which each associated stage was identified...).
3. The number of passages used for the bone marrow derived cells should be added to the methods.
4. Methods, page 11, second line: ‘..upstream sense and downstream sense primers...’
5. In the Results section, there is a reference to figure 2A then to 3A followed by 2B and 3B. The authors should consider changing the figures in a way that will enable their sequential mention in the text.
6. The following sentence appears in the Discussion (First paragraph, page 21) ‘Our data added to the previous studies strongly support that Src kinase activity is the main target for dasatinib in MSC differentiation process’. However, the experiments described in the manuscript do not address this question, and the only evidence for that is an experiment performed with another Src inhibitor that produced similar results. As this additional experiment is not part of the current manuscript there is no data here to show that the main target of dasatinib in MSC is Src.

Other points for consideration:
The manuscript adheres to the standards for reporting and the writing is acceptable. The abstract accurately describes the study, but the conclusions and the title are somewhat overstating the results. The only indications for stimulation of osteoblast differentiation by dasatinib are in figure 7; on day 14 calcium concentrations are higher with dasatinib and on day 7 ALP activity is higher. The gene expression data is not quantitative and therefore unreliable. The inhibition of RANKL occurs only in the absence of DAG on day 7 and the suggestion of an indirect inhibition of osteoclastogenesis is therefore based on a single experimental point.

**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 that I have no competing interests