Reviewer’s report

Title: Alterations in Osteoclast Function and Phenotype Induced by Different Inhibitors of Bone Resorption - Implications for Osteoclast Quality

Version: 1 Date: 29 September 2009

Reviewer: Rachel r Davey

Reviewer’s report:

This study by Neutzsky-Wulff et al investigates the role of various osteoclast inhibitors representative of inhibitors of acidification and proteolysis on osteoclast-mediated degradation of both organic and inorganic bone matrix in an in vitro osteoclast cell system. While the information provided by this study is potentially of significant interest to the osteoclast biology field, the validity of the data cannot be assessed in its current format.

Major Compulsory Revisions:
The manuscript contains a number of grammatical errors which require correction. There are also a number of sentences that lack punctuation. These errors are too numerous to list, however as an example, page 6, line 14 “Lack of or dysfunctional cathepsin K is the cause of the disease pycnodysostosis, a bone disease which also displays increased bone mass.

The conclusion sections of the abstract and discussion are very general and would benefit from the inclusion of a statement summarizing the key findings of the paper. Also, what significance do the differences in the mode of action of the different types of osteoclast inhibitors tested in the current study have on the progression and treatment of bone disease?

The methods section lacks significant detail. There is no mention of how many times each experiment was repeated or how many replicates were included for all experiments. This also includes the gel depicted in Figure 4B. If this is a representative gel, then it should be stated as such in the figure legend. As such the validity of all of the data presented in this paper and therefore the conclusions that have been made based on this data cannot be assessed.

In addition the methods section should include what concentration of the inhibitors were tested and the rationale for these concentrations. Also, how were mature osteoclasts (page 9, line 15) defined?

The results section also lacks detail and is very speculative. The speculation would best be moved to the discussion section.

Specific Points:
P values should be included.

It is stated that E64 potently reduced organic resorption by 80%. At what concentration was this magnitude of inhibition observed?
On page 16, line 8 it is stated that co-treatment of cells with E64 and GM6001 slightly increased the % resorbed bone area, but no significantly. If there is no significant difference in % bone resorbed area, then there is no effect. This statement requires correction.

It is not clear why TRACP activity data is only provided for the experiments described in 5. As significant changes in TRACP activity were observed in other experiments albeit mild, they are still significant and should therefore be presented for all experiments.

It should state that Figure 6 is the author’s proposed mechanism of osteoclast action.

Minor Essential Revisions:
The statement on page 6, lines 1 and 2 require a reference.

Parts of the introduction are repetitive and would benefit from some careful editing.

The reader is required to refer back and forth to various figures throughout the results section. As such, it would be better to separate the figures to allow them to be presented in sequential order.

Please clarify the source of the data not shown that is referred to on page 22 of the discussion. Is this the authors’ own observation or that of a colleague?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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