Reviewer's report

Title: Bone Growth during Rapamycin Therapy in Young Rats

Version: 4 Date: 28 October 2008

Reviewer: H Anderson

Reviewer's report:

General Comment: This report presents important new data supporting earlier studies that indicate a negative effect of rapamycin therapy on young, growing rat tibial growth plates, causing impaired long bone growth in 2 week-old rats (but not in 4 week-old rats). The findings presented are in good agreement with earlier studies, and present interesting new data, showing rapamycin's effect on individual cells' expression of several growth factors and changes in cellular structure induced by rapamycin. Most of the comments and criticisms from an earlier review have been adequately responded to. However, there are still a few questions regarding labeling of figures that present histochemical and in situ hybridization detected changes caused by Rapamycin. (see below.)

Specific comments:

Page 4, line 4. The changed sentence provided here for this revision is confusing, and should be reworked to indicate clearly the equivalent bone ages in rats versus in children.

Page 11, Fig. 2: This figure could be better labeled to reduce confusion between the microscopic effects of rapamycin on collagen II expression (upper left panel), versus on H-4 protein expression (middle left panel), and on mTOR expression (lower left panel), by labeling each panel in larger letters at the top of each panel. For example, the upper left panel would say "Type II collagen expression" written at the top of it and the left middle panel would say "H-4 protein expression", written over the top of it, etc. The same labeling pattern could easily be used with the analytical panels on the right of Fig. 2.

Page 11, third paragraph, Fig. 3: The same pattern of labeling as suggested for Fig. 2 could be used to clarify Fig. 3, and also Figs. 4, 5, 6 and 7.

Page 13, paragraph 3, Fig. 7, lower left panel. These microscopic images, that are said to show a decreased number of TRAP-staining chondro/osteoclasts in rapamycin-treated animals at 2 and 4 weeks of age, are not convincing, and do not support the numerical, osteoclast count data presented in the lower right panel. In fact the photomicrographs seem to suggest that there are more TRAP-positive osteoclasts in growth plates and metaphyses of the rapamycin-treated rats.

Level of interest: An article of outstanding merit and interest in its field
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.