Reviewer's report

Title: Host-Derived RANKL is Responsible for Osteolysis in C4-2 Human Prostate Cancer Xenograft Model of Experimental Bone Metastases

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Reviewer: Janet Rubin

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General

This group has set out to determine whether tumor expression of RANKL is responsible for the osteolysis associated with tumor growth in bone. They have shown that an antibody to human RANKL (huRANKL MAb) prevents hypercalcemia caused by twice weekly injections of huRANKL to an equal extent as OPG, but does not prevent the growth and bone destruction of CaP (C4-2) injected intratibially. From this they conclude that tumor derived RANKL is not significantly involved in bony destruction. While they have used some thoughtful approaches and have done extensive work, they do not entirely answer the question as to the source of biologically significant RANKL.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors should explain why they did not use SCID male mice in Figure 1. In 1A male BDF mice are used to show that huRANKL causes hypercalcemia and that this can be prevented by 10 mg/kg huRANKL MAb. If they know that huRANKL MAb does the same thing in SCID mice, they should cite the data. In 1B they use female BALB/c mice to show that OPG inhibits bone turnover, decreasing TRACP 5b, but that huRANKL MAb does not have the same effect. They are probably correct in concluding that huRANKL MAb performs in a species specific manner, but it would be helpful to know if mouse RANKL MAb decreased TRACP 5b.

2. The nice data in Figure 2 show RANKL is highly expressed in the C4-2 tumor, and that huRANKL MAb did not prevent tumor growth/establishment as assessed by PSA. It would have been helpful to show that OPG did or did not have an effect on this particular tumor, similar to the partial effect of OPG in another CaP line they have studied (Kiefer 2004), and that Zhang et al 2001 have shown in a related tumor line (C4-2B). At the very least they should comment on their experience.

3. The data in Figure 3 shows that tumor growth and bony destruction caused by C4-2 intratibial injection is not prevented by huRANKL MAb. These data are matched by the histology in Table 1. The difference in osteoclast number in the treatment group may not be biologically significant.

4. It is unclear what conclusions they can make with the data in figure 4. They do not shown a true control for either TRACP 5b or for calcium, i.e., a age/sex/strain matched mouse without a tumor – is that because the TRACP 5b and calcium in the tumored “control” is unchanged? Furthermore, if osteolysis can occur in the absence of increased TRACP 5b (and/or calcium), bone destruction seen in fig 3 may simply not depend on RANKL from the host or the tumor: might the skeleton simply fail in the face of a rapidly expanding lesion? It may be that the authors could argue this point, especially if they have data showing that OPG could prevent the bone destruction.. The second possibility, which they discuss on page 18, is that the huRANKL MAb might not work very well. If the huRANKL MAb did not “demonstrate strong immunoreactivity…on the C4-2 cells”, was this the right approach?

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

none
Discretionary Revisions (which the author can choose to ignore)

none

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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.