Successful treatment of primary and secondary malignant bone tumors in many cases is still limited. Among various variables, the microenvironment is one of the most important ones determining survival and growth of tumors. For the development of new therapeutic strategies, a better understanding of host-tumor interactions might be extremely helpful.

In the literature, there are described plenty of most various experimental in vivo and ex vivo models all having specific advantages and disadvantages limiting the predication of results achieved by these models. The present study aims to introduce a newly developed in vitro co-culture model which might be able to open new insights into host-tumor interactions between the bone as a site of orthotopic implantation and primary and secondary malignant bone tumors. By using neonatal femurs of mice in combination with various malignant tumor cells implanted as a co-culture on 6-well culture plates the authors claim to be able to monitor the expression, release and regulation of paracrine factors during the interaction of a bone explant and tumor cells with the advantage (vs. in vivo models) of better accessibility of the microenvironment for sampling and analysis of paracrine factors and the advantage (vs. in vitro models) of the presence of the three-dimensional architecture of the bone and the multiple cell interactions present in their bone-tumor microenvironment.

Major Compulsory Revisions

1. Since a newly developed model is presented, the methods should be explained and discussed more in detail:
   a) Are the mice used inbred or not? Are they immuno-competent or not and if yes - are there any immunological reactions to expect since human tumors are used, too?
b) What was the exact method of stripping femurs? Which kinds of soft tissues remained in contact to the bone (muscles, tendons, periost ect.)? In figure 1B it is obvious that there is a lot of soft tissue sticking to the femur.

c) In line 296 it is written "... the outer cell layers of the femurs ...". In a defined experimental model it has to be clear what the exact components of the "outer cell layers" are.

d) Was the preparation of femurs performed under a microscope? How femurs were prevented of drying during the preparation?

e) How long did it take between sacrificing the mice, preparation of femurs and implantation of the femurs into the 6-well culture plates? Was this procedure standardized for all experiments?

f) In lines 275 and 448 it is stated, that the femurs used are similar to adult bone. This is wrong. Here we have the typical bone of a new born growing organism. About half of the bone is cartilage. Ossification centers in epiphyses are still missing (see Fig. 1B). The composition of cellular, mineral and other components of these bones is completely different to "adult" bone. The occurrence of metastases - in contrast to primary bone tumors - would be extremely rare during this stage of development.

g) The method of flushing of the marrow cavity of dissected femurs with PBS (lines 398ff) should be described in detail.

2. An exact experimental protocol should be provided. In the present study, there are no exact including or excluding criteria for experiments. Sometimes it remains unclear how many experiments were performed per experimental group (see line 791 e.g. “6-9 wells/group”). In the figures for example number of wells/group easily could be included.

3. The statistical analysis has to be completely redone and described in detail. If the number of femurs per treatment and time point is tree for example (see line 761), it is doubtful whether a statistical test at all should be performed - and if yes, then a non-parametric analysis should be performed. Data in this case should be provided as single values or as median with maximal and minimal range and not as mean±SEM or SE.

4. The 250µm optical slice (line 299) should be defined in detail since the bars on images shown in Fig. 2A and B are 0.38 and 0.15mm whereas the parts of femurs shown seem to have the same size.

5. The methods which were performed in lines 309 to 316 should be explained in detail and the results of these experiments should be presented in detail.

6. There is no change in numbers of apoptotic cells inside the femurs between day 2 and 4 (see Fig. 2F). It remains unclear when the increase of apoptosis starts. Since the time point when apoptosis starts may limit the experiments to study secreted factors in the present co-culture model further experiments should be added regarding to this issue. In contrast to lines 315-318, eventually experiments should be limited to less than 48h.
7. With regard to the results, the conclusion of the present study is too descriptive. The conclusion that the model might be useful in studying the role of secreted factors like MCP-1 in bone cancer pain by expanding the components is too speculative since nerves, pain etc. were not investigated at all.

8. The limitations of the model should be discussed more in detail.

9. The title is promising to much in contrast to the findings of the study

Minor Essential Revisions

1. In the abstract it might be added that the femurs are from mice and that murine and human cancer cell lines are used.

2. SI units should be used as abbreviations “h” instead of “hs” e.g.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests