For many years bisphosphonates are an established treatment of bone metastases. Drugs of this substance class are able to reduce the number of skeletal complications by inhibiting osteoclast mediated bone destruction. Numerous experimental and clinical studies have shown that interventional bisphosphonate treatment can reduce the occurrence of pathologic fractures, bone pain, hypercalcemia and the need for radio therapy and surgery. Animal experiments and initial clinical experience with preventative application of bisphosphonates seem to be very promising and more beneficial than a palliative application. Investigations into the use of bisphosphonates are now directed to understand the effects of a preventative treatment on tumor osteolysis. The 3-D microstructure of trabecular bone is one factor influencing the mechanical behavior of trabecular bone. Most recently new imaging techniques for the determination of the alteration of the 3-D structure of trabecular bone have been introduced. In a recent study, we demonstrated that micro-computed tomography (µCT) is able to provide 3-D parameters of bone mass and trabecular structure in an animal model for tumor-induced bone loss. We found significant differences in 3-D morphometric parameters between the tumor-bearing bones and contralateral controls.(1) In the present experimental study, we investigated the effect of a preventative application of a bisphosphonate on the 3-D microstructure of bone in a rat model for tumor-induced osteolysis. The research questions were: 1. Does an interventional treatment with a bisphosphonate affect the tumor-induced destruction of the 3-D structure of trabecular bone? 2. Is a preventative treatment with a bisphosphonate more beneficial than interventional application in preserving the 3-D structure of trabecular bone in tumor osteolysis?

**Methods and Materials:** Forty five Sprague-Dawley rats were randomly assigned to three study groups. In the first group (T), Walker 256 tumor cells were implanted into the left femur without treatment. The second group (PTI) first underwent a daily pretreatment with ibandronate (0.003 mg·kg⁻¹) for 26 days, then tumor cells were implanted and the daily treatment was continued until sacrifice. The third group (TI) received the daily ibandronate treatment only after tumor-cell implantation. Twenty-eight days after tumor implantation all animals were killed, femora harvested and cleaned of soft tissue. As in the recent study we employed a desk-top µCT imaging system (µCT 20, Scanco Medical; 304 µm, nominal resolution) to assess three-dimensional densitometric and architectural parameters in small animal whole bones. We scanned a total of 90 specimens (left and right femora). For each specimen a total of two-hundred micro-tomographic slices were taken starting from a reproducible anatomical landmark right behind the boundary of the condyles covering a region of 6 mm of the distal femur. Two regions were considered for the quantitative analysis, Bone mineral content (BMC) based on the measured bone volume (BV) and the assumption of a constant tissue density was calculated as the product of BV and the assumption of a constant tissue density was analyzed for a volume of interest (VOI) in the metaphysis consisting of both cortical and trabecular bone (ctBMC), and for a sphere consisting of trabecular bone only (tBMC). Architectural indices were only calculated for the spherical VOI according to standard definitions used in histomorphometry. Bone volume density (BV/TV) and trabecular plate number (Tb.N) were determined directly from the binarized 3-D images. From these primary indices, bone surface density (BS/TV), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) were derived using a parallel plate model. All 90 specimens were analyzed using both visual assessment and quantitative histomorphometry. A Wilk-Shapiro test for normality was conducted on all parameters. Analysis of variance (ANOVA) with SNK post-hoc analysis was performed to determine if the measurements differed significantly (p<0.05).

**Results:** The µCT analysis revealed greater values for ctBMC and tBMC in the PTI group and TI group compared to the T group (Table 1).

<table>
<thead>
<tr>
<th>Group Comparison</th>
<th>Percentage Differences between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention/Intervention vs Tumor</td>
<td>*114%</td>
</tr>
<tr>
<td>Intervention vs Tumor</td>
<td>*174%</td>
</tr>
<tr>
<td>Prevention/Intervention vs Intervention</td>
<td>*14%</td>
</tr>
</tbody>
</table>

The morphometric analysis in the trabecular volume revealed results on the architectural changes which were in accordance with the changes seen in bone density on the apparent level. BV/TV was significantly higher in the PTI and TI groups compared to the T group by 133% and 106%, respectively. These results are most likely due to a significant increase in Tb.Th in the PTI and the TI group compared to the T group by 211% and 95%, respectively. Additionally, Tb.Sp decreased in the PTI and TI group compared to the T group by -74% and -73%, respectively. PTI values were 13% greater for BV/TV and 60% for Tb.Th compared to TP. BS/TV and Tb.N were not significantly affected indicating that treatment worked mostly on existing trabeculae (increased width) rather than the formation of new trabeculae.

**Discussion:** The aim of the present study was to investigate the effect of a bisphosphonate on bone density and 3-D bone structure in a rat model for tumor-induced osteolysis. This study demonstrated that a daily interventional treatment with ibandronate starting after the onset of tumor growth in bone preserves bone mass and the 3-D structure in the model applied. Ibandronate given as a combination of preventative and interventional treatment resulted in greater density and structural parameters compared to untreated tumor-control group. Compared to the interventional control group we found a more beneficial effect on density parameters and trabecular thickness. Our results support other experimental studies investigating the effect of preventative treatment with bisphosphonates. Krempien et al.(3) reduced the WCS 256 tumor destruction of bone by a pretreatment with clodronate and Kestenuik et al. showed a three time higher trabecular volume in animals pretreated with pamidronate as compared to their controls. In conclusion, although both treatment schedules resulted in significantly greater bone mass and structural parameters compared to no treatment, combined preventative and interventional treatment provides the best protection against tumor-mediated osteolysis.

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**Session 16 – Tumors - Esplanade Ballroom 306-308, Sun 4:30 PM - 6:00 PM**


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**The Effect of a Preventative Treatment with Ibandronate on the Microstructure of Bone in Tumor Osteolysis**

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