INTRODUCTION
Breast, prostate, lung, and renal cancers are the most common primary tumours to metastasize to bone, with the vertebral column being the most frequently affected site. Spinal metastases can compromise the mechanical integrity of the spine and thereby increase the risk of pathological fractures. While breast cancer has historically been associated with osteolytic spinal metastases, the incidence of mixed osteolytic/osteoblastic disease in the spine secondary to breast cancer has increased with widespread use of bisphosphonates.

Therapeutic approaches for spinal metastases should aim to both reduce tumour burden and restore structural stability. Photodynamic therapy (PDT) is a minimally invasive treatment that involves administration of a photosensitizer, which preferentially accumulates in malignant cells. Once excited by light, the photosensitizer produces highly reactive singlet oxygen that causes cell toxicity and death. PDT has the potential to be applied to the spine by delivering light to the vertebral body by modifying a minimally invasive technique originally developed for vertebroplasty. Previous work in an osteolytic rat model (MT1 human breast cancer cells) showed that a single PDT treatment with the photosensitizer benzoporphyrin derivative monoacid ring A (BPD-MA) effectively ablates tumour and improves vertebral structural properties.

In particular, vertebral structural properties were improved by minimizing the osteolytic bone destruction. It is unknown how PDT would affect the surrounding bony tissue in a more clinically representative mixed osteolytic/osteoblastic preclinical model. Thus, the purpose of this study is to assess the efficacy of PDT in a rat model of mixed osteolytic/osteoblastic spinal metastases.

METHODS
The effects of PDT were evaluated in 36 5-6 week old female athymic nu/nu rats, randomly assigned to four treatment groups: 1) healthy untreated controls (H-Ctrl); 2) healthy PDT-treated (H-PDT); 3) tumour-bearing untreated controls (T-Ctrl); and 4) tumour-bearing PDT-treated (T-PDT). Vertebral metastasis was induced in groups 3 and 4 by intracardiac injection of luciferase-transfected Ace-1 canine prostate cancer cells into the left heart ventricle (day 0). Institutional animal care committee approval was obtained for all procedures (University Health Network, Toronto, Canada).

PDT was administered on day 14 with 1.0 mg/kg of BPD-MA photosensitizer (verteporfin, Visudyne; Novartis, Dorval, Canada). Under fluoroscopic guidance, an optical fiber was inserted percutaneously adjacent to the target second lumbar vertebra, L2. The photosensitizer was activated 15 minutes later with 75 J of light energy delivered through a 690 nm diode laser at a power output of 100 mW. Half of the rats received 10 mg/kg calcine green as a first bone fluorochrome label (day 14) and 90 mg/kg xylene orange as a second bone fluorochrome label (day 17). Animals were euthanized on day 21.

All spines were imaged with a specimen micro-computed tomography (µCT) scanner (GE Explore Locus; GE, Fairfield, CT) at a 17.4 µm/voxel resolution, at 80 kVp and 90 µA. The following stereological parameters were quantified (Amira 5.2; TGS, Berlin, Germany): trabecular bone volume fraction (BV/TV), trabecular architecture (thickness (Tr.Th), spacing (Tr.Sp), number (Tr.N)), and trabecular bone surface to bone volume fraction (BS/BV). Half of the samples were then processed for decalcified/undecalcified histology and analysed using Genie Pro image analysis (Aperio Technologies Inc.). Undecalcified samples were stained with Goldner’s Trichrome to quantify osteoid volume (OV/BV), while decalcified samples were stained with haematoxylin and eosin (H&E) to evaluate cell morphology; parathyroid hormone-related protein (PTHrP; in tumour-bearing samples) to visualize tumour cells in the vertebrae; tartrate-resistant acid phosphatase (TRAP) to quantify osteoclast activity per bone volume (OC/BV) and number of large (>3 nuclei) and small (≤2 nuclei) osteoclasts (LOc and SOc); and Picrosirius Red S to distinguish morphology; parathyroid hormone-related protein (PTHrP; in tumour-bearing samples) to visualize tumour cells in the vertebrae; tartrate-resistant acid phosphatase (TRAP) to quantify osteoclast activity per bone volume (OC/BV) and number of large (>3 nuclei) and small (≤2 nuclei) osteoclasts (LOc and SOc); and Picrosirius Red S to distinguish

Table 1. Remodeling Properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>H-Ctrl</th>
<th>H-PDT</th>
<th>T-Ctrl</th>
<th>T-PDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC/BV (%)</td>
<td>0.50 ± 0.08</td>
<td>0.48 ± 0.10</td>
<td>3.44 ± 1.55a</td>
<td>0.24 ± 0.06b</td>
</tr>
<tr>
<td>L.Oc (-)</td>
<td>10 ± 1</td>
<td>7 ± 1</td>
<td>17 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>N.Oc (-)</td>
<td>18 ± 2</td>
<td>15 ± 2</td>
<td>26 ± 14</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>OV/BV (%)</td>
<td>0.86 ± 0.12</td>
<td>0.45 ± 0.90</td>
<td>4.31 ± 0.92a</td>
<td>3.00 ± 1.90b</td>
</tr>
</tbody>
</table>

a = p<0.05 for T-Ctrl vs H-Ctrl; b = p<0.05 for H-PDT vs H-Ctrl; c = p<0.05 for T-PDT vs T-Ctrl.

RESULTS
Ace-1 metastatically-involved vertebrae exhibit significantly decreased structural properties compared to untreated controls; Ace-1 untreated vertebrae exhibit a 10% decrease in BV/TV, an 11% reduction in Tb.N, and 23% increased trabecular spacing compared to untreated controls. In addition to areas of osteolytic destruction (Figure 1B), several areas of osteoblastic lesions were observed through evidence of new bone formation (white arrows), notably on the periosteal surface.

Figure 1. Transverse Sections from µCT (A) healthy control; (B) Ace-1 untreated; (C) Ace-1 PDT-treated
determined using multivariate analysis of variance (ε=0.05).

Figure 1. Transverse Sections from µCT (A) healthy control; (B) Ace-1 untreated; (C) Ace-1 PDT-treated

Decreased structural properties are due to increased osteoclast activity/size: Ace-1 untreated vertebrae exhibit an almost 7-fold increase in OC/BV compared to untreated controls, as well as 70% and 44% more large and small osteoclasts, respectively (Table 1). Ace-1 vertebrae also exhibited increased osteoid volume compared to healthy controls, again, most strikingly on the periosteal surface.

DISCUSSION
Ace-1 mixed osteolytic/osteoblastic spinal metastases exhibit enhanced yet decoupled bone remodelling, leading to decreased vertebral bone quality. PDT effectively destroys tumour tissue, while increasing bone structural properties and decreasing osteoclastic bone resorption. This finding is similar to what we have previously observed in our pure osteolytic (MT1) rat model. PDT does not further stimulate new unmineralized bone (osteoid) formation in mixed osteolytic/osteoblastic metastases, which it appears to do in healthy bone. As such, the effect of PDT on mixed osteolytic/osteoblastic bone metastases appears to be through a suppression of osteoclastic resorption as opposed to triggering new bone formation. PDT effects on bone are more prominent in tumour-bearing vertebrae, possibly due to increased vascularisation and subsequent photosensitizer concentration. Overall, this study has further motivated PDT as a viable and appealing treatment for spinal metastasis secondary to breast cancer.

REFERENCES

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