INTRODUCTION:
The skeleton is a common site of metastases for a number of different cancers. They include lung, breast, prostate, kidney, thyroid cancers and multiple myeloma. It has been reported that in 70% of patients with metastatic breast cancer, bone metastases will develop [1]. Cancer cells in bone disrupt normal bone remodelling, resulting in the case of breast tumour cells in osteolytic lesions causing pain and fracture. Electrochemotherapy (ECT) refers to the combination of electroporation and administration of hydrophilic anticancer drugs for the local treatment of cutaneous and subcutaneous tumoural lesions of any histotypes [2]. Electroporation induces transient permeabilization of cell membrane by applying short and intense electric pulses; electroporation allows low permeant or non permeant anticancer drugs diffusion into the cytosol increasing their cytotoxicity by order of magnitude [3]. Previous studies showed that electroporation of osteoblasts lining the trabeculae as well as of osteocytes can be achieved with no alteration of the mineral component or mechanical competence (microhardness) of the bone tissue [4]. No experience has been ever reported on ECT application in bone metastases. Purpose of our study was to assess the efficacy of ECT in the treatment of in vivo rat mammary adenocarcinoma MRMT-1 bone metastases.

METHODS:  
In vitro model. Cultured MRMT-1 cells were tested for sensibility to electroporation, to the anti cancer drug bleomycin, and to the combination of electroporation and bleomycin; cell proliferation and viability were assessed using the tetrazolium salt WST-1 assay.  
Animal model. The study was performed according to the Italian law on animal experimentation. The protocol was approved by the ethical committee of the Rizzoli Orthopaedic Institute and by the Italian Ministry of Health. In vivo experiments envisaged that 3,000 MRMT-1 cells were inoculated in the proximal tibia of rats to induce osteolytic bone metastases. Seven days post implantation rats were treated with bleomycin i.v. (15,000 IU/m²) alone or with bleomycin plus electroporation. All animals were evaluated weekly by X-ray and at sacrifice, 21 days post-implantation, by Micro-CT and histology analysis.  
Electrical treatment. Electroporation was performed using the Cliniporator (IGEA, Carpi, Italy); different protocols of 100 µs square-wave electric pulses were tested in vitro; 1 or 2 sequences of 8, 100 µs, electric pulses at 1000 V/cm were used for in vivo experiments.  
Statistical Analysis. Comparisons between groups were performed using Student’s T test and p<0.05 level was considered significant.

RESULTS SECTION:  
In vitro data showed that, compared to bleomycin alone, cell viability was significantly decreased when bleomycin was associated to electroporation p<0.001 (Figure 1). In vivo cell inoculation resulted in spontaneous fracture of the tibia within 21 days in control animals and in rats treated with bleomycin alone. Cancellous bone appeared shattered, and cancer cells invaded the surrounding area. The trabecular bone tissue was almost completely replaced by tumor tissue (Figure 2A). Moreover, hematopoietic cells from the bone marrow were replaced by carcinoma cells (Figure 2B). In contrast, all animals responded to ECT treatment: trabeculae maintained a normal structure and morphology (figure 2C) exhibiting typical bone tissue lamellae and interspersed lacune with osteocytes (figure 2D). No osteolytic lesions were visible. In addition micro CT analysis of rat tibias 21 days following MRMT-1 cells inoculation, showed a high bone volume with the formation of new trabeculae in the tibiae treated with ECT (Figure 3).

DISCUSSION:  
This results demonstrate that ECT is feasible and effective in the treatment of rat breast cancer bone metastases. Following preclinical studies from our group, which have shown no alteration of bone mineral structure after ECT, a phase I-II clinical trial studying electrochemotherapy for the treatment of osteolytic bone metastasis has been approved and patients enrolled. No complications were reported and fast patient’s recovery was assessed demonstrating feasibility and safety of ECT on bone metastases [5]. Our study shows, for the first time, the efficacy of ECT on bone cancer. Micro CT analysis reveals normal bone tissue in the treated tibias. Moreover evidence of new bone formation in the treated areas indicates that ECT may also protect from the risk of pathological fracture aiding to complete lesion resolution. Taken all together our data lays encouraging bases for new clinical studies and good potential clinical responses

SIGNIFICANCE:  
ECT is a quick, safe and less invasive operative technique for the treatment of cutaneous and subcutaneous tumour nodules. In the management of bone metastases, ECT might bring a great benefit to patients minimising the need of surgical treatment for small lesions. Doing so the here proposed innovative, therapeutic approach would ultimately aim to reduce the metastatic patients discomfort improving their quality of life.

REFERENCES:  
5. Bianchi G et al. EMSOS meeting, [Poster], May 2010