INTRODUCTION:
We reported that third-generation bisphosphonates (BPs), YM175 and YM529 significantly inhibit the in vitro growth of murine osteosarcoma cell lines in a time- and dose-dependent manner by preventing prenylation of small GTPases and inducing apoptosis (51st ORS). In this study, we changed the drug under study to zoledronic acid (ZOL), another third-generation BP, which is as potent as YM529 at inhibiting bone resorption in vivo even though their R² substituents differ. To investigate the effect of BPs further, we examined the way to augment the effect of BPs by combination with other anti-tumor drugs.

MATERIALS AND METHODS:
We used third-generation BPs, ZOL and other six anti-tumor drugs; three of which are commonly used for treating osteosarcoma (doxorubicin, cisplatin, and methotrexate) and another three that are undergoing clinical trial or have been used in vitro (STI571, paclitaxel, and gemcitabine). We used murine osteosarcoma cell lines, MOS and its multidrug-resistant subclone, MOS/ADR, and another murine osteosarcoma cell line, LM8, which was established from the murine Dunn osteosarcoma cell line and has high metastatic potential to lung. (1)

(1) Concurrent exposure to ZOL and other anti-cancer drugs
Cell viability was evaluated by MTT assay. The IC₅₀ values were obtained by using the nonlinear regression program CalcuSyn (Biosoft, Cambridge, United Kingdom). Then, osteosarcoma cells were treated with 6 concentrations (0.25, 0.5, 0.75, 1.0, 1.5 or 2.0 x IC₅₀) of ZOL alone, an anti-cancer drug alone and the ZOL/anti-cancer drug combination in 96-well plates. Each plate was evaluated individually for efficacy. The fraction affected (Fa) (i.e., Fa of 0.25 is equivalent to 75% viable cells) and the combination index (CI) were calculated with CalcuSyn (Biosoft). This method enables quantification of synergism (CI<1) and antagonism (CI>1) at different dose and effect levels. CI calculations were made under the assumption that the mechanisms of drug action were not mutually exclusive.

(2) Sequential exposure of cells to ZOL and other anti-cancer drugs
LM8 cells were incubated with 15 µM ZOL for 24 hours. After the LM8 cells were washed thrice in PBS, the second anti-cancer drug was added to the respective wells. After a further 48 hours the rate of growth inhibition was evaluated by MTT assay. Data from 3 independent experiments were collected. P values of less than 0.05 were considered statistically significant and were derived from two-sided statistical tests.

RESULTS:
(1) Cytotoxic interactions from concurrent exposure of cells to ZOL and other anti-cancer drugs
The Cl-versus-Fa plots in Figure 1 reveal the effects on MOS and LM8 cell growth from exposure to a combination of ZOL and 6 other anti-cancer drugs. The Cls at Fa 0.5 and at Fa 0.8 were less than 1.0 ± 1 SD in both osteosarcoma cell lines indicating that the effects of combination are synergistically, when combined with paclitaxel or gemcitabine (Figure 1E,F). In contrast, when combined with methotrexate, the Cls at all fractions consistently exceeded or were with 1.0 ± 1 SD in both osteosarcoma cell lines, indicating that combination with methotrexate produces antagonistic rather than additive effects (Figure 1C).

DISCUSSION:
The aim of this study was to determine whether the anti-tumor effect of BPs can be strengthen when it was combined with other anti-tumor drugs.

In this present study, we found that combination of ZOL with doxorubicin or cisplatin resulted in additive or synergistic growth inhibition of osteosarcoma cell lines. These results may have therapeutic application, particularly for enhancing the efficacy of a drug that cannot be administered at higher dosages because of its toxicity. Therefore, we may be able to combine ZOL with the present osteosarcoma chemotherapy regimes. In addition, the anti-growth activities of paclitaxel and gemcitabine were completely augmented by ZOL in both concurrent and sequential treatment regimes. Therefore, ZOL may have therapeutic application in enhancing the efficacy of these drugs such that their effective doses against other malignant neoplasms, such as lung cancer and ovarian cancer, can be reduced. Moreover, the simultaneous administration of ZOL and paclitaxel or gemcitabine clearly and synergistically inhibited the proliferation of a P-glycoprotein-overexpressing osteosarcoma cell line. Although we did not actually determine whether the combination overcomes the multi-drug resistance system, these results appear to offer benefits to osteosarcoma patients.

In conclusion, our results indicate that the combination of ZOL with doxorubicin, cisplatin, paclitaxel, or gemcitabine may be more effective against murine osteosarcoma than the use of any of these drugs alone.