ABSTRACT INTRODUCTION:
Telomere studies in human carcinomas have been extensively reported for prognostic utility and effective methods for targeting telomerase therapy has been described, but efficacy of telomerase inhibitor remained unknown in sarcoma cells. Telomeres are specialized structures containing unique (TTAGGG), repeats at the ends of chromosomes which are thought to be important in the protection and replication of chromosomes. Telomerase contains an RNA-dependent DNA polymerase, which is a cellular reverse transcriptase, and stabilizes telomere length by adding DNA repeats onto telomere ends. Sarcomas are distinct from carcinomas in that many of them use alternative lengthening of telomeres (ALT) mechanisms. Many types of sarcoma have been reported to have elongated telomeres consistent with ALT in the absence of telomerase activity, indicating the existence of non-telomerase-based ALT mechanisms for telomere maintenance. The telomere maintenance mechanism by ALT has recently reported as a mechanism of telomere lengthening in sarcoma cells or sarcoma cell lines, HOS (telomerase-), SaOs2 (telomerase-, ALT+), MG63 (telomerase+,- ALT+) and U2OS (telomerase-, ALT+), which were treated with TMPyP4 after which cell growth, telomerase activity, telomere length were investigated.

METHODS:
Evaluation of telomerase activity was performed by fluorescein-based telomeric repeat amplification protocol (TRAP) assay. Telomere Length was analyzed with Southern blotting. Cell proliferation was examined by MTT assay. The cells were incubated in the presence of a TMPyP4 concentration of 50 or 100 μM for 48h or 96h. The significance of differences between groups was evaluated by the paired t-test. The level of significant was set at p < 0.05.

RESULTS SECTION:
1. TMPyP4 for 96 hours at dose of 50 μM inhibited telomerase activity in telomerase positive HOS (p = 0.0001) (Figure 1) and SaOs2 cells (p = 0.0003), but not in MG63 cells (p = 0.109).
2. Treatment with TMPyP4 for 96 hours significantly induced telomere shortening in HOS cells (50 μM: p = 0.0045, 100 μM: p = 0.0021), SaOs2 cells (p = 0.0029, p = 0.0029) (Figure 2), and MG63 cells (p = 0.0217, p = 0.0185), but not induce telomere shortening in U2OS cells (p = 0.103). Telomere length was 7.22 ± 0.18 kb of the control, and 5.52 ± 0.02 kb with treatment at 50 μM, and 5.60 ± 0.54 kb with treatment at 100 μM in HOS cells. Telomere length was 11.9 ± 1.19 kb of the control, and 4.88 ± 0.14 kb with treatment at 50 μM, and 4.00 ± 0.68 kb with treatment at 100 μM in SaOs2 cells. Telomere length was 13.8 ± 0.52 kb of the control, and 10.7 ± 0.33 kb with treatment at 50 μM, and 11.6 ± 0.45 kb with treatment at 100 μM in MG63 cells. Telomere length was 39.9 ± 2.19 kb of the control, and 39.7 ± 2.20 kb with treatment at 50μM, and 37.6 ± 1.13 kb with treatment at 100 μM in U2OS cells.
3. Treatment with TMPyP4 at doses of 50 μM for 48 or 96 hours significantly inhibited the growth of HOS cells (48 hours: p = 0.0045, 96 hours: p = 0.0001), SaOs2 cells (p < 0.0001, p = 0.0003), and U2OS cells (p = 0.00003) (Figure 3, 4) but not in MG63 cells with treatment for 96 hours (p = 0.0069, p = 0.109).

DISCUSSION:
G-quadruplex-interactive agents TMPyP4 reported to be a telomerase inhibitor require long-term culture to show telomerase inhibition and telomere shortening. In the present study, treatment with TMPyP4 significantly induced inhibition of telomerase activity and telomere shortening in HOS and SaOs2 cells. However, TMPyP4 did not induce inhibition of telomerase activity in MG63 cells, although it induced significant telomere shortening. Treatment with TMPyP4 significantly inhibited the growth of HOS cells and SaOs2 cells, but not in MG63 cells. Previous report revealed that low telomerase activity was not sufficient to prevent telomere shortening, which in turn gives rise to ALT in the osteosarcoma cell line Saos-2, may indicating telomerase is not necessary to immortalize osteosarcoma cells. However, our results may suggest that both telomerase activity loss and sufficient telomere shortening are necessary to inhibit cell growth in telomere positive osteosarcoma cells.

Average telomere length after treatment was 5.52-5.60 kb in HOS, 4.00-4.88 kb in SaOs2 and 9.89-10.7 kb in MG63 cells. In many cells, when telomeres become critically short, further cell division is blocked and cells reached to as replicative senescence. These results also may suggest that telomere shortening less than 6 kb is sufficient to senescence or inhibition of cell growth. In terms of U2OS cells which is telomerase negative with extremely longer telomere (39.9 ± 2.19 kb), treatment with TMPyP4 did not induced telomere shortening but significantly inhibited the cell growth. Our results of telomerase negative extremely longer ALT phenotype U2OS cells may be due to presence of unknown antitumor effects of telomerase inhibitor to ALT cells or may be related to DNA damage including telomere dysfunction through G-quadruplex stabilization, independent on telomere length. Our results may indicate G-quadruplex interactive agents such as TMPyP4 are novel adjuvant therapy for osteosarcoma patients in the near future. Sarcoma cells have heterogeneous characteristics in telomere biology, therefore, further study is necessary to clarify the mechanisms of antitumor activity by G-quadruplex target therapy.