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The novel HSP90 inhibitor STA-12-1474 exhibits biologic activity against canine osteosarcoma cell lines

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Heat shock protein 90 (Hsp90) is a molecular chaperone that promotes correct folding, maturation, and stabilization of numerous client proteins including kinases, hormone receptors, and transcription factors. Hsp90 inhibition is an attractive therapeutic strategy as Hsp90 clients are frequent contributors to the oncogenic process, notably EGFR family members, AKT, Bcr-abl, Kit, and Met. In particular, Met is an attractive target for therapeutic intervention in osteosarcoma (OSA) as Met over-expression in human OSA tumor cell lines is associated with a more aggressive phenotype and poor survival. The purpose of this study was to evaluate the potential therapeutic utility of a novel Hsp90 inhibitor, STA-12-1474 for the treatment of OSA. Canine and human OSA cell lines as well as normal canine osteoblasts were treated with STA-12-1474 at various concentrations followed by CyQuant evaluation to assess cell viability. In addition, cell lines were treated with STA-12-1474 or PF2362376 (a novel small molecule Met inhibitor) followed by AnnexinV-FITC/PI staining and flow cytometric analysis for apoptosis. Hsp70, Hsp90, p-Met, Met, p-Stat3, Stat3, p-Akt, and Akt levels were detected by western blot analysis following treatment with STA12-1474 or PF2362376 and stimulation with recombinant human HGF. Finally, doxorubicin, a chemotherapy agent used in the treatment of OSA, was evaluated alone and in combination with STA-12-1474. Our data demonstrate dose and time dependent inhibition of OSA cell proliferation and viability in the presence of biologically relevant concentrations of STA-12-1474. Additionally, STA-12-1474 induced apoptosis in treated cells which was significantly greater than that induced by PF2362376 treatment. Normal canine osteoblasts were 10-100 fold less sensitive than canine OSA tumor lines to STA-12-1474. Inhibition of Hsp90 by STA-12-1474 promoted upregulation of Hsp70 in all OSA cell lines; this was not seen with PF2362376 exposure. In addition, STA-12-1474 treatment resulted in downregulation of p-Met and Met as well as p-Stat3, p-AKT and Akt, while only loss of p-Met was observed in PF2362376 treated cells. Total STAT3 levels remained unchanged following STA-12-1474 treatment indicating that an upstream activator of STAT3 rather than STAT3 itself is a client of Hsp90 in these OSA cell lines. STA-12-1474 or doxorubicin alone inhibited OSA cell viability; however, there was no evidence of synergistic activity when both drugs were used together. These data support STA-12-1474 as a promising therapy for the treatment of OSA and indicate the need for further analysis of therapeutic strategies involving standard chemotherapeutics in combination with Hsp90 inhibitors.