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Sorafenib and Palbociclib Combination Regresses a Cisplatinum-resistant Osteosarcoma in a PDOX Mouse Model

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Abstract. *Background/Aim: Recurrent osteosarcoma is a recalcitrant disease; therefore, an improved strategy is urgently needed to provide therapy. In order to develop a novel strategy for this disease, our lab has developed a patient-derived orthotopic xenograft (PDOX) mouse model for osteosarcoma. The combination of sorafenib (SFN) and palbociclib (PAL) was shown to be effective of hepatocellular carcinoma. However, whether this combination is efficacious on osteosarcoma has not been reported. The aim of this study was to determine the efficacy of the SFN and PAL combination on a cisplatinum (CDDP)-resistant osteosarcoma PDOX model. Materials and Methods: Osteosarcoma PDOX models were randomly divided into five treatment groups: untreated-control, CDDP, SFN, PAL and the combination of SFN and PAL. Results: Of these agents, the SFN-PAL combination significantly regressed tumor growth, and enhanced tumor necrosis with degenerative changes in the osteosarcoma PDOX. Conclusion: The SFN-PAL combination is an effective treatment strategy for osteosarcoma and therefore holds promise for clinical efficacy.*

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Cisplatinum (CDDP) is widely used against osteosarcoma (1). However, recurrent or advanced osteosarcoma is a recalcitrant disease resistant to CDDP and other first-line therapies (2). Sorafenib (SFN) is an inhibitor of several tyrosine protein kinases that has been approved for the treatment of various cancers (3-5). Palbociclib (PAL) is a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor that regulates the cell-cycle, inhibits cell growth and suppresses DNA replication (6-8).

CDK inhibitors are preferentially used in combination with other drugs that convert temporary cell-cycle inhibition to permanent growth arrest or cell death (7). SFN and PAL have been shown to have synergistic effects against hepatocellular carcinoma and pancreatic carcinoma (6, 7). However, the efficacy of SFN and PAL combination for osteosarcoma has not been reported.

In the present report, using a patient-derived orthotopic xenograft (PDOX) model of osteosarcoma, we demonstrate for the first time that the SFN-PAL combination could overcome CDDP-resistance.

Materials and Methods

Mice. Athymic nu/nu nude mice (AntiCancer, Inc., San Diego, CA, USA) were used in this study (9, 10). Detail protocols for animal handing, breeding, anesthesia and surgery have been presented previously (9-13). All experiments were performed at AntiCancer, Inc. The protocols used in the mouse study were approved by the Institutional Animal Care and Use Committee (IACUC) (9). All the studies were conducted according to the principles and procedures described in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1 (9).

Patient-derived tumor. A fresh biopsy sample from a 14-year-old boy with pelvic conventional osteosarcoma was used (10). Sample preparation and its subcutaneous implantation in nude mice has been previously described (10). Before biopsy this patient did not

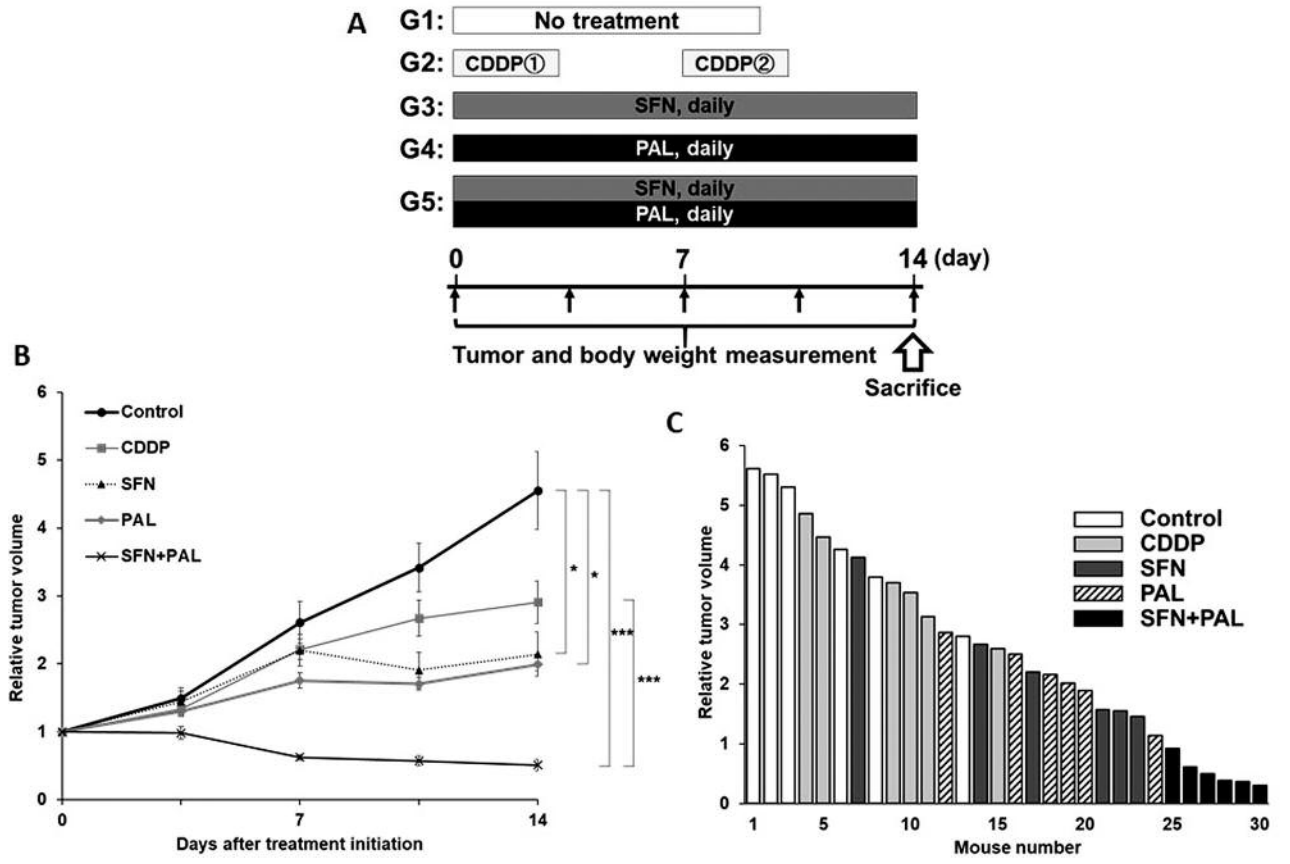

Figure 1. *Treatment schema and efficacy of drugs. (A) Treatment protocols in the osteosarcoma PDOX model. (B) Relative tumor volume of the osteosarcoma-PDOX model. The relative tumor volume is the tumor volume at any time during treatment compared to the tumor volume at the initiation of treatment. Untreated control (p=0.56); SFN (p=0.02); PAL (p=0.01); and SFN-PAL (p<0.001). (C) Waterfall plot of relative tumor volume (day 14/ day 0) for each mouse. N=6 mice/group. *p<0.05; ***p<0.001. Error bars: ±SEM.*

receive any chemotherapy or radiotherapy. The patient has provided a written informed consent as part of a UCLA Institutional Review Board approved protocol (IRB#10-001857) (11).

Developing the osteosarcoma PDOX model. The detailed procedures for mouse anesthesia, and orthotopic implantation of tumors into the mouse distal femur have been previously described (12, 13). The detailed protocols for skin incision and tumor fragment implantation were performed as described previously (12, 13).

Treatment protocols in the osteosarcoma PDOX model. The osteosarcoma PDOX mouse models were randomly divided into five groups. Each group contained six mice. The mice were treated with the following drugs for two weeks (Figure 1A) as previously described (10): G1-untreated control; G2-CDDP (6 mg/kg, intraperitoneal (i.p.) injection, once a week) alone; G3-SFN (30 mg/kg, oral, daily) alone; G4-PAL (100 mg/kg, oral, daily); G5-SFN + PAL. Treatment started when all tumors reached 100 mm^3 volume. Tumor length, width, volume, and mouse body weight were measured as described previously (10). Data are presented as mean ± standard error of the mean (SEM).

Histological analysis. Fixation of fresh tumor samples, sectioning and staining were performed as described previously (10).

Ethical approval. The study was performed under AntiCancer Inc. IACUC (Institutional Animal Care and Use Committee)-approved protocol. The patient has provided a written informed consent as part of a UCLA Institutional Review Board approved protocol (IRB#10-001857) (11).

Statistical analysis. All statistical analyses were performed as described previously (10). The Shapiro-Wilk test was used for normal distribution (10). To verify the homogeneity of variances across groups, the Bartlett's test was performed. For the parametric test for inter-group comparison, one-way ANOVA with Tukey HSD for *post-hoc* analysis was performed (10) and to compare the means between two related groups, the paired *t*-test was performed (10). All *p*-values were two sided and *p*-values of less than 0.05 were regarded as statistically significant.

Results

Testing efficacy of drugs on the osteosarcoma-PDOX. Osteosarcoma PDOX models treated with CDDP showed no difference in comparison to untreated controls (p=0.56). While SFN alone or PAL alone significantly, but moderately,

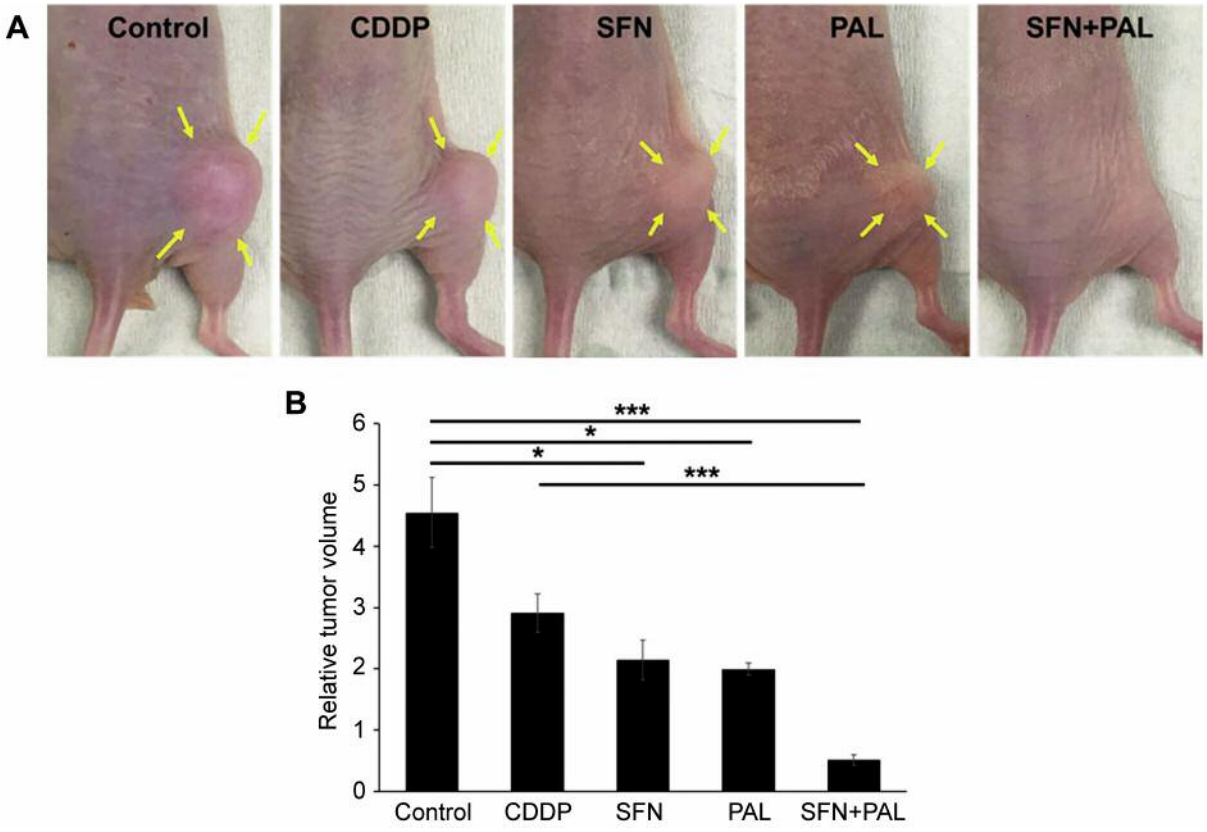

Figure 2. *Efficacy of drugs on tumor volume. (A) Representative images of osteosarcoma PDOX mouse models at the end of each treatment. Arrows indicate the clear margin of the tumors. (B) Bar graphs show relative tumor volume of each treatment group on day 14. N=6 mice/group. *p<0.05; ***p<0.001. Error bars: ±SEM.*

inhibited the growth of osteosarcoma PDOX compared to the untreated control (p=0.02 and p=0.01, respectively). In contrast, the combination of SFN-PAL dramatically regressed the osteosarcoma PDOX tumor (p<0.001). Of these drugs, only the SFN-PAL combination was more efficacious compared to CDDP (p<0.001). (Figures 1B, C and 2A, B).

Histology of the osteosarcoma PDOX. Histological analysis showed that the untreated control tumor had viable highly-dense cancer cells that were pleomorphic and spindle-shaped. Untreated control tumors also had many mitotic events and osteoid laid down in a lace-like pattern among tumor cells (10). Tumors treated with a single dose of CDDP, SFN, or PAL had viable tumor cells, although the cancer-cell density was relatively low as compared to the untreated control. However, tumors treated with the SFN-PAL combination presented cancer necrosis with non-viable cells and frequent occurrence of degenerative scars in the stroma. These results suggested that this combination is efficacious in the osteosarcoma PDOX models (Figure 3A).

Effect of treatment on body weight. As compared to initial body weight, in the untreated control group, the final body weight of mice was significantly increased (p=0.02). However, no significant difference in body weight among the other groups was noted (Figure 3B). In addition, no other observable side effects or animal death in any group were noted.

Discussion

Here, for the first time, we found that the SFN-PAL combination regressed tumor in an osteosarcoma PDOX model. The synergistic effect of the SFN-PAL combination treatment in cancer treatment has been investigated only in hepatocellular and pancreatic carcinomas (6, 7), but not in osteosarcoma. SFN and PAL alone have shown potential efficacy for osteosarcoma (3, 4, 14). Most osteosarcomas have upregulated VEGFR, PDGFR, or MAPK activation, and inhibition of MAPK by SFN has been shown to be highly effective in osteosarcoma preclinical models (4). It has been shown that CDK4 is highly expressed in osteosarcoma tissues and cell lines (14). An elevated CDK4 expression in immunohistochemical analysis of

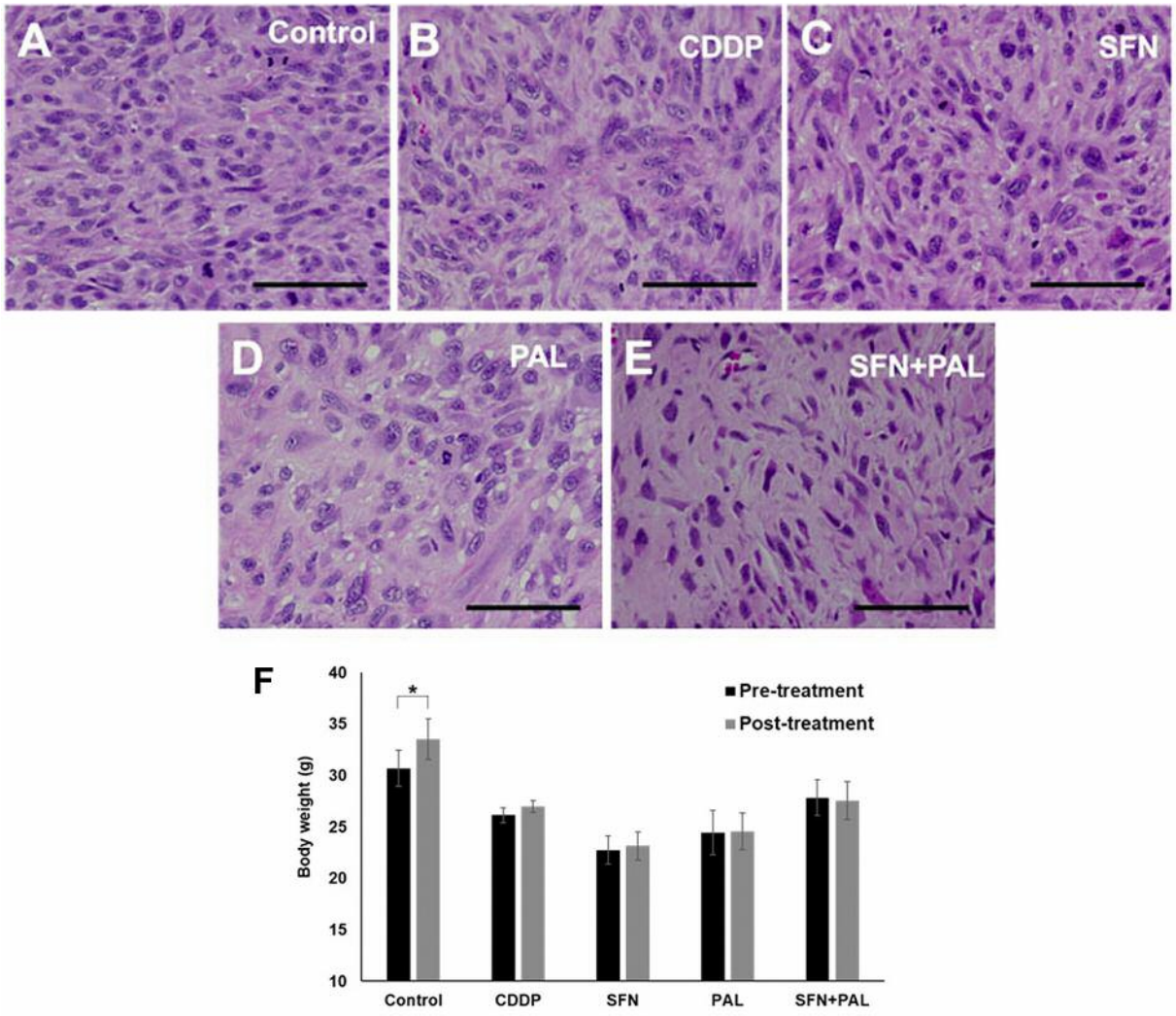

Figure 3. *Tumor histology and mouse body weight. (A) Untreated control, (B) CDDP, (C) SFN, (D) PAL, and (E) SFN + PAL. Scale bars: 100 μm. (F) Bar graphs show mouse body weight in untreated-control and each treatment group at pre- and post-treatment times. *p<0.05. Error bars: ±SEM.*

osteosarcoma patients correlated with the development of metastasis and poor prognosis, suggesting that PAL may be effective for osteosarcoma (14). Here, we showed the moderate efficacy of SFN and PAL as single usage, and the high efficacy of the SFN-PAL combination on the cisplatinum-resistant osteosarcoma PDOX model for the first time. The ability of SFN-PAL combination to regress PDOX tumor suggests clinical efficacy.

Our laboratory developed PDOX mouse models of various cancers (9-13, 15-19). Previously, we have shown that our PDOX model is more patient-like compared to subcutaneous patient-derived xenograft (PDX) models (15, 16). In addition, we have demonstrated that the PDOX model maintains the original histological and molecular features after xenograft in nude mise (17, 18). PDOX models provide the opportunity to test unique personalized treatment options for sarcoma patients.

In conclusion, this study identified an effective treatment strategy using the SFN-PAL combination for recalcitrant osteosarcoma which holds promise of clinical efficacy.

Conflicts of Interest

AntiCancer Inc. uses PDOX models for contract research. TH, NS, MK, HO, NY, KH, HK, SM, KI and RMH are or were unsalaried associates of AntiCancer Inc. There are no other competing financial interests.

Authors' Contributions

Conception and design: TH and RMH. Acquisition of data: TH, NS, KM, HO, NM, KH, HK, SM, and KI. Analysis and interpretation of

data: TH, NS, KM, HO, NM, KH, HK, SM, KI, SPC, MB, SRS, HT, and RMH. Writing, review, and/or revision of the manuscript: TH, RMH, HT, and SRS.

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