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Combination of Trabectedin With Oxaliplatinum and 5-Fluorouracil Arrests a Primary Colorectal Cancer in a Patient-derived Orthotopic Xenograft Mouse Model

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Abstract. *Background/Aim: In the present study, we aimed to determine the efficacy of trabectedin (TRAB) combined with oxaliplatinum (OXA)+5-fluorouracil (5-FU) on a colorectal cancer (CRC) patient-derived orthotopic xenograft (PDOX) mouse model. Materials and Methods: A patient CRC tumor previously established in nude mice was implanted subcutaneously in transgenic green fluorescence protein (GFP)-expressing nude mice. Harvested tumor fragments were transplanted orthotopically in non-transgenic nude mice. Mice were randomized into three groups: Group 1 (G1), untreated-control; Group 2 (G2), OXA+5-FU; Group 3 (G3), TRAB+OXA+5-FU. Tumor width, length, and mouse body weight were measured twice a week. Results: Both treatment groups inhibited tumor growth compared to the untreated control group. The combination of TRAB, OXA and 5-FU was significantly more efficacious than OXA+5-FU and arrested tumor growth. No significant changes were observed in body-weight in any of the three groups. Conclusion: TRAB, OXA and 5-FU combination has clinical potential for this and other CRC patients.*

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Key Words: Colorectal cancer, trabectedin, oxaliplatinum, 5-fluorouracil, combination therapy, PDOX.

Currently-available treatment strategies for colorectal cancer (CRC) mainly include surgical resection, radiotherapy and adjuvant chemotherapy. Oxaliplatinum (OXA)-based chemotherapy after surgical resection is one of the most frequently used therapeutic strategies (1). OXA combined with 5-fluorouracil (5-FU) has increased the survival rate in patients with CRC (2). However, a large proportion of patients with CRC receiving chemotherapy recur and develop chemo-resistant metastasis (3). Therefore, more effective therapies are needed for metastatic CRC.

To accomplish this goal, we have developed a patient-derived orthotopic xenograft (PDOX) nude mouse model for many tumor types (4-13). To test the response of various chemotherapies on individual CRC patients, we have also developed CRC PDOX nude-mouse models and orthotopic cell line (13-21).

Trabectedin (TRAB) has been used for treating many cancer types (22, 23). TRAB is effective against many tumors resistant to alkylating agents (24). However, there are few studies where it has been shown that TRAB is active in CRC. Stevens *et al*., (25) have reported that several colorectal cell lines are highly resistant to cisplatinum and sensitive to TRAB. Izbicka *et al*. (23) and Twelves *et al*. (26) have shown that CRC patients could benefit from TRAB. In the present study, we show that TRAB is efficacious on a CRC PDOX mouse model in combination with OXA+5-FU.

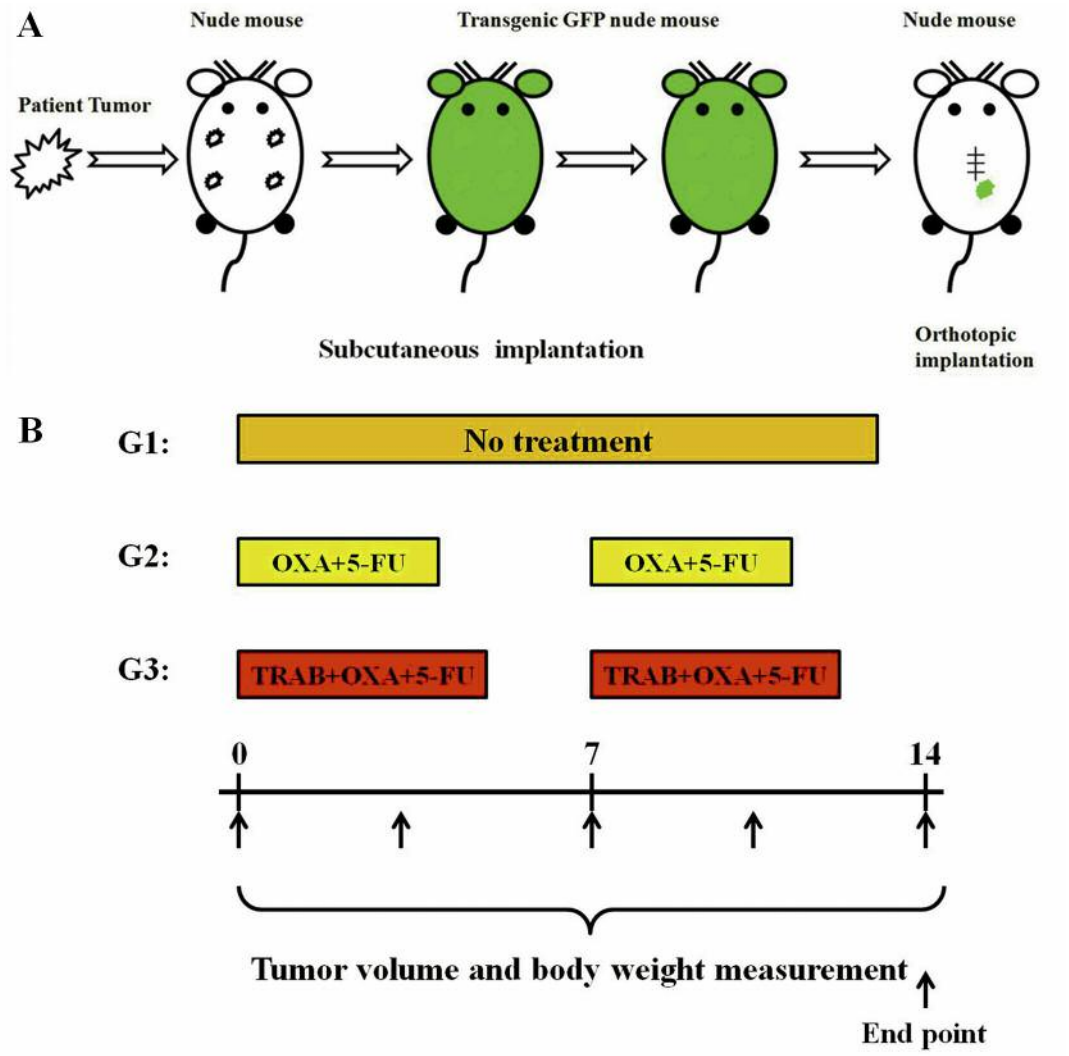

Figure 1. *Establishment of a patient-derived orthotopic xenograft (PDOX) model and drug treatment schema. (A) Schematic illustration of the surgical orthotopic implantation (SOI) for establishment of imageable PDOX (iPDOX) models of human colorectal cancer. (B) Treatment regime and quantitative drug efficacy. Treatment protocol. G1: untreated control (n=7); G2: treated with OXA+5-FU (OXA 6 mg/kg+5-FU 50 mg/kg; i.p., weekly for 2 weeks); G3: treated with TRAB+OXA+5-FU (TRAB 0.15 mg/kg, i.v. and OXA 6 mg/kg+5-FU 50 mg/kg; i.p., weekly for 2 weeks). All treated mice were sacrificed on the endpoint day, and tumors were resected for further histological evaluation.*

Materials and Methods

Mice. Athymic nu/nu nude mice 4- to 6-weeks and transgenic green fluorescence protein (GFP) expressing athymic nu/nu mice were obtained from AntiCancer Inc. (San Diego, CA, USA). All surgical procedures and imaging were performed in accordance with an AntiCancer Inc. Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study, and in accordance with the principles and procedures outlined in the National Institutes of Health Guidelines for the Care and Use of Animals under Assurance Number A3873-1 (11). Mouse housing, feeding, anesthesia and surgical procedures were performed as described in previous publications (11, 27). The mice were humanely sacrificed based our previous publication (27).

Patient-derived tumor. The primary tumor was previously obtained from a patient with CRC in the Division of Surgical Oncology, University of California, San Diego (UCSD). The patient did not receive any chemotherapy or radiotherapy prior to surgery. Fresh tumor tissues were obtained from the patient at surgery with informed patient consent and Institutional Review Board (IRB) approval. The CRC PDOX mouse model (iPDOX) was established using the surgical orthotopic implantation (SOI) technique, as previously reported (14).

Establishing the imageable CRC PDOX model. CRC samples were cut into 5 mm^3 fragments and implanted subcutaneously in nude mice. The resulting CRC tumors grown in non-transgenic nude mice were harvested, cut into 5 mm^3 fragments, and implanted

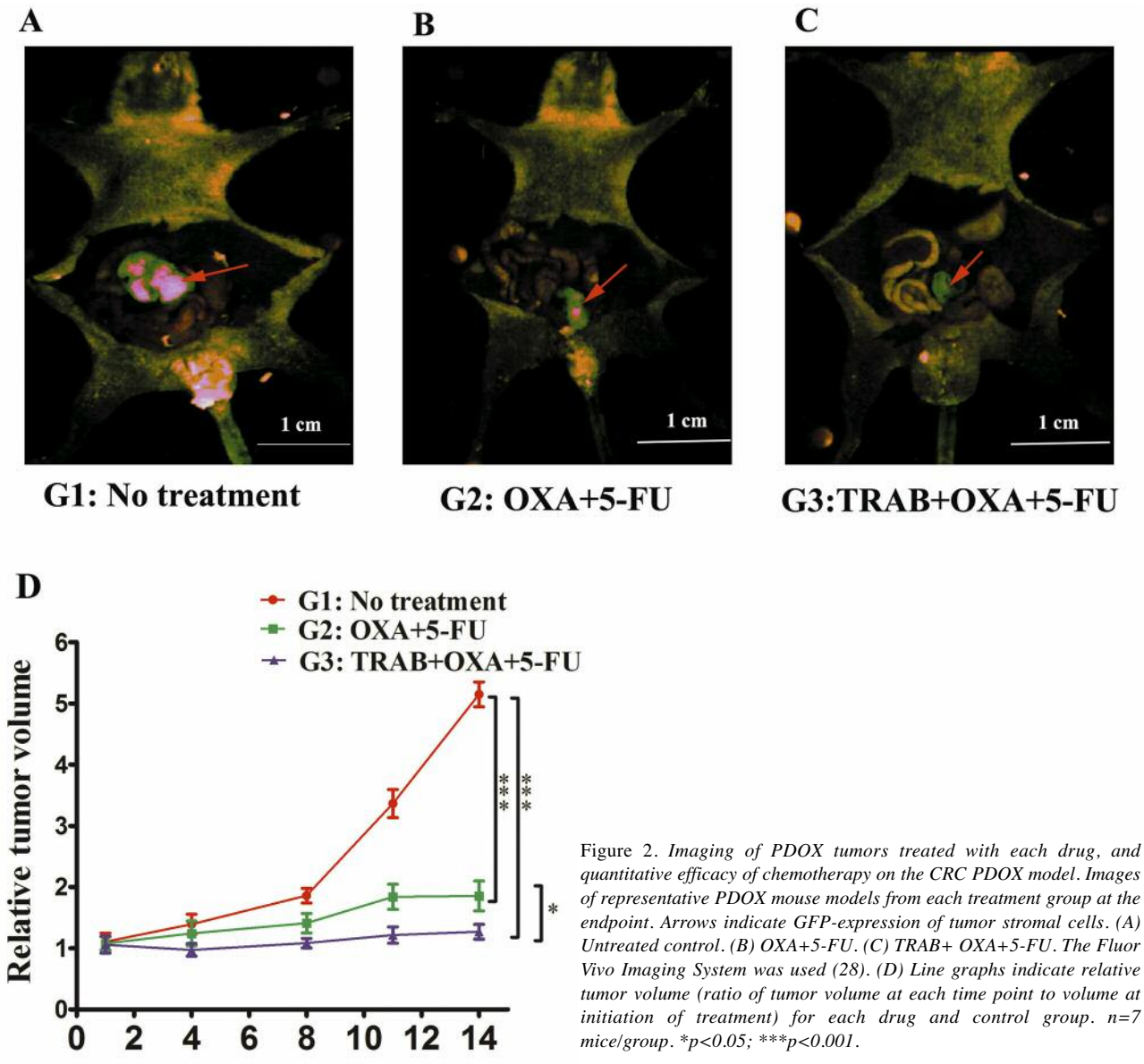

Figure 2. *Imaging of PDOX tumors treated with each drug, and quantitative efficacy of chemotherapy on the CRC PDOX model. Images of representative PDOX mouse models from each treatment group at the endpoint. Arrows indicate GFP-expression of tumor stromal cells. (A) Untreated control. (B) OXA+5-FU. (C) TRAB+ OXA+5-FU. The Fluor Vivo Imaging System was used (28). (D) Line graphs indicate relative tumor volume (ratio of tumor volume at each time point to volume at initiation of treatment) for each drug and control group. n=7 mice/group. *p<0.05; ***p<0.001.*

subcutaneously in green fluorescence protein (GFP)-expressing transgenic nude mice. After two passages in GFP-expressing nude mice, tumors stably containing GFP-expressing stromal cells were harvested and cut into 5 mm^3 fragments. After non-transgenic nude mice were anesthetized with a ketamine mixture, an approximately 1-cm skin incision was made at the middle of the abdomen. 8-0 nylon sutures were used to implant tumor fragments onto the cecum. Wounds were closed using 6-0 nylon sutures as described before (14). The schematic diagram to establish a CRC PDOX model is shown in Figure 1A.

Treatment study design. Six weeks after orthotopic implantation of CRC-GFP tumors, the abdomen of the PDOX mice was opened to observe tumor growth. The PDOX mice were randomized into 3 groups (7 mice/per treatment group) by measuring tumor size: Group 1 (G1), control group (no treatment); Group 2 (G2), OXA+5-FU (OXA 6 mg/kg; 5-FU 50 mg/kg; i.p., weekly for 2 weeks); Group 3 (G3), TRAB+ OXA+5-FU (0.15 mg/kg, i.v.; OXA 6 mg/kg; 5-FU 50 mg/kg; i.p., weekly for 2 weeks) (Figure 1B). Tumor length, width and mouse body weight were measured twice a week. Tumor volume was calculated by the following formula: tumor volume (mm^3)=length (mm)×width (mm)×width (mm)×1/2. Data are presented as mean±SD. Mice were imaged with the Fluor Vivo Imaging System (INDEC Biosystem, Santa Clara, CA, USA) (28).

Histological analysis. Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Four-μm tissue sections were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was carried out according to a standard protocol.

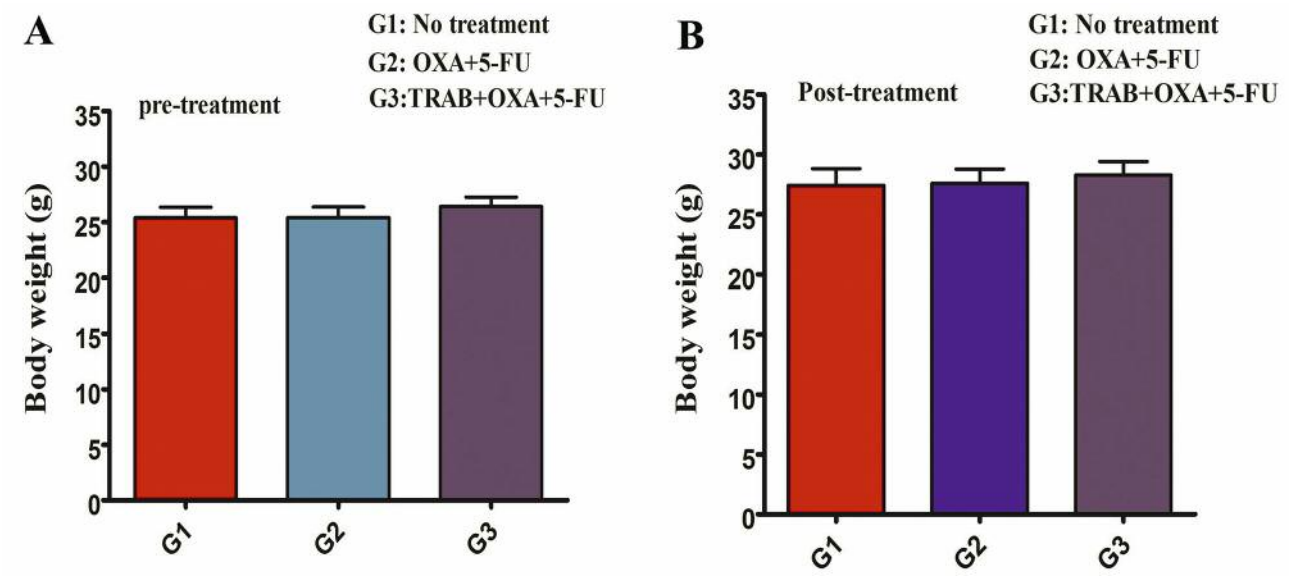

Figure 3. *Effect of drugs on mouse body weight. Bar graph shows body weight in each group at pre-treatment (A) and 2 weeks post-treatment (B). There were no significant differences between any group.*

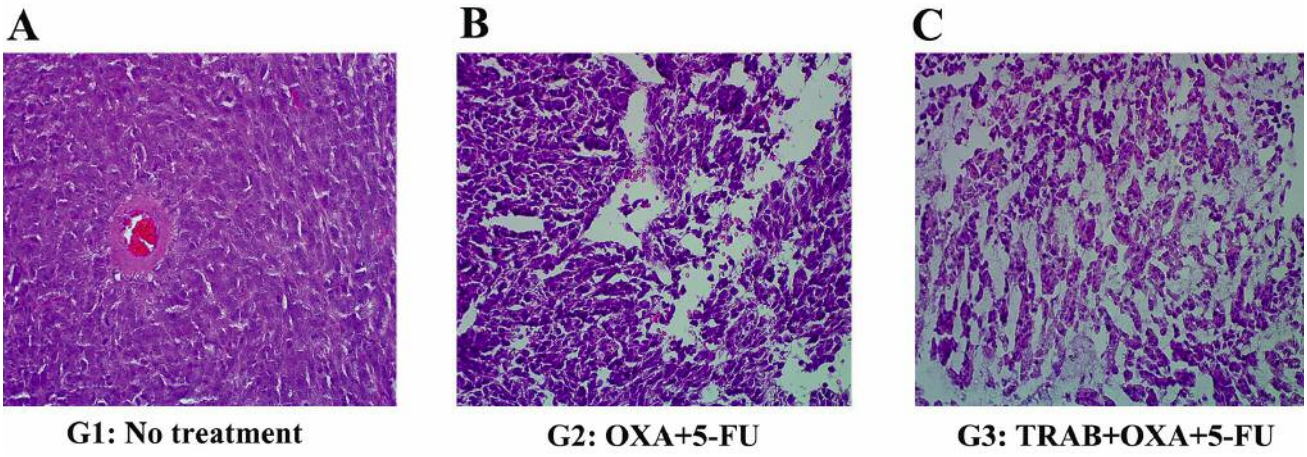

Figure 4. *Histology of colorectal cancer PDOX mouse model in treated and untreated tumors. Hematoxylin and eosin (H&E)-stained sections of the (A) Untreated control group. (B) OXA+5-FU treated group, and (C) TRAB+OXA+5-FU treated group. Microscope magnification is 200×.*

Histological visualization was performed with a BHS system microscope and images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada) (29).

Statistical analysis. All statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc. La Jolla, CA, USA). Data were analyzed by ANOVA with Tukey's *post-hoc* test when more than two groups were compared. The data were expressed as the mean±SD. A p-value of <0.05 was considered to be statistically significant.

Results

Drug efficacy. To test the efficacy of each drug in the CRC PDOX mouse models, six weeks following orthotopic implantation, mice with tumors were randomized into three groups to initiate treatment (Figure 1B). The tumor volumes at the endpoint of the experiment were: G1 untreated control, 514.8±20.3 mm^3 (Figure 2A); G2 OXA+5-FU, 185.4±24.3 mm^3 (Figure 2B); G3 TRAB+OXA+5-FU, 127±12 mm^3

(Figure 2C). The control group (G1) tumors grew more than four times larger after 2 weeks compared to the time of the initial treatment (tumor-volume ratio= 4.65±1.48). In the OXA+5-FU group (G2), at the endpoint, there was significant inhibition in tumor growth compared to the control group (tumor-volume ratio=1.67±1.77, $p<0.001$). The TRAB+OXA +5-FU combination group (G3) arrested tumor growth (tumor-volume ratio=1.15±0.88, $p<0.001$, compared to the control) (Figure 2D). The TRAB+OXA+5-FU (G3) group resulted in a greater inhibition of tumor growth than the OXA+5-FU group (G2) ($p<0.05$) (Figure 2D).

Effect of drug treatment on body weight. There was no significant difference observed in body weight in all three groups (Figure 3A, B).

Tumor histology. Histologically, the un-treated control tumor mainly comprised viable cancer cells (Figure 4A). In the tumors treated with OXA+5-FU, cancer-cell density was lower than that of the control (Figure 4B, C). The strongest efficacy was observed with the combination of TRAB+OXA+5-FU. In this group, cancer cell density was the lowest among all three groups (Figure 4C).

Discussion

OXA+5-FU is first line treatment for CRC (30, 31). Although OXA combined with 5-FU showed a response in the majority of CRC cases, virtually all the responses are incomplete because patients have recurrences. In order to develop an effective curative strategy, the combination of TRAB+OXA+5-FU was tested to see whether it is efficacious in inhibiting the growth of a CRC PDOX mouse model. We found that the combination of TRAB+OXA+5-FU was more effective compared to the OXA+5-FU regimen.

TRAB has been previously shown to re-sensitize resistant cells in some tumors (32). TRAB has also been shown to be efficacious in sarcoma (33-36), recurrent ovarian cancer (37), metastatic breast cancer (38), juvenile myelomonocytic leukemia, and chronic myelomonocytic leukemia (39) and we found that TRAB was effective in PDOX models of sarcoma (40-43).

Although, some studies have shown that TRAB may be effective for CRC (23, 25, 26), no studies have examined the efficacy of the combination of TRAB+OXA+5-FU on CRC. In the present study, we showed that TRAB+OXA+5-FU was highly effective against a PDOX mouse model of CRC, suggesting the use of this combination in the clinic.

Conflicts of Interest

GWZ, YS and RMH are unpaid affiliates of Anticancer Inc. MZ and QH are employees of AntiCancer Inc. AntiCancer Inc. uses PDOX models for contract research. The authors declare no conflicts of interest regarding this study.

Authors' Contributions

GWZ, and J-XY designed the study, modified the figures and wrote draft manuscript; GWZ performed the experiments; MZ, QHH and YS gave help in the experiments; SRS and RMH revised the manuscript. All Authors approved the final manuscript.

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