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Original Articles

Olaratumab combined with doxorubicin and ifosfamide overcomes individual doxorubicin and olaratumab resistance of an undifferentiated soft-tissue sarcoma in a PDOX mouse model

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ABSTRACT

Olaratumab (OLA), a monoclonal antibody against platelet-derived growth factor receptor alpha (PDGFRα), has recently been used against soft-tissue sarcoma (STS) combined with doxorubicin (DOX), with limited efficacy. The goal of the present study was to determine the efficacy of OLA in combination with DOX and ifosfamide (IFO) on STS. Undifferentiated soft-tissue sarcoma (USTS) from a striated muscle of a patient was grown orthotopically in the right biceps femoris muscle of nude mice to establish USTS patient-derived orthotopic xenograft (PDOX) model. USTS PDOX tumors were treated with OLA alone, DOX alone, DOX combined with IFO, OLA combined with DOX or IFO, and OLA combined with DOX and IFO. Tumor size and body weight were measured during the 14 days of treatment. Tumor growth was arrested by OLA combined with DOX and IFO. Tumors treated with OLA combined with DOX and IFO had the most necrosis. The present study demonstrates the power of the PDOX model to identify the novel effective treatment strategy of the combination of OLA, DOX and IFO for soft-tissue sarcomas.

1. Introduction

High-grade soft-tissue sarcomas (STS) including undifferentiated soft-tissue sarcoma (USTS) are recalcitrant neoplasms in need of novel therapeutic strategies [1]. The American Cancer Society estimated that approximately 13,000 new cases and about 5000 deaths from soft tissue sarcoma (STS) will occur in the United States in 2018 [2]. The treatment for STS is mostly dependent on the tumor grade, size, metastatic sites and histological subtype [3,4]. Surgical resection is the only curative treatment option for USTS because it is resistance to radiotherapy and most chemotherapy [5]. Several chemotherapy drugs such as aldoxorubicin, amrubicin, eribulin, trabectedin, tivozanib, doxorubicin (DOX), ifosfamide (IFO) and gemcitabine (GEM) have been in clinical trials for the treatment of STS [6–12]. STS is usually treated with DOX-containing regimens as first-line therapy, but with limited efficacy [1,13]. IFO in combination to DOX has been shown to more effective against STS compared to DOX alone and in combination chemotherapy for STS, but still with limited efficacy [1,14,15].

In STS, overexpression of platelet-derived growth factor receptor alpha (PDGFRα) indicates poor prognosis [16–18]. Olaratumab (OLA) is a recombinant human IgG1 monoclonal antibody that is directed against the PDGFRα [4,19]. OLA inhibits the activation of PDGF-AA, PDGF-BB and PDGF-CC receptors and downstream signaling [20,21]. OLA combined with DOX increased median overall survival compared

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to DOX alone in patients with STS [22].

Because of the heterogeneity and recalcitrance of USTS, precise individualized therapy is needed. In this regard, we have developed the patient-derived orthotopic xenograft (PDOX) mouse models of cancer using the technique of surgical orthotopic implantation (SOI) [23–29]. We previously established a PDOX nude mouse model of DOX-resistant USTS using SOI in the right biceps femoris muscle [29].

The aim of the present study was to identify more effective combinations with OLA for STS using the PDOX model of USTS.

2. Materials and methods

2.1. Mice

Athymic nu/nu nude mice (AntiCancer, Inc., San Diego, CA, USA), 4–6 weeks old, were used. Experimental procedures and data collection were carried out as previously described [29]. To minimize any suffering of the animals, anesthesia and analgesics were used for all surgical experiments. The mouse investigations presented here were carried out using an AntiCancer, Inc. Institutional Animal Care and Use Committee (IACUC) protocol specifically approved for this study as previously described [30] and as per as the principles and procedures provided in the National Institute of Health (NIH) Guide for the Care and Use of Animals under Assurance Number A3873-1 [30].

2.2. Patient-derived tumor

A 62-year-old female previously diagnosed, with undifferentiated sarcoma not otherwise specified in her left upper arm, underwent surgical resection at Department of Surgery, University of California, Los Angeles (UCLA) [29]. The patient did not receive any chemotherapy or radiotherapy prior to surgery. Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board approved protocol (IRB#10–001857) [30].

2.3. Surgical orthotopic implantation (SOI) for establishment of a USTS PDOX model

A schematic illustration of SOI for establishment of USTS PDOX model is shown in Fig. 1A. The tumor was cut into small fragments and initially implanted into nude mice subcutaneously [29]. After three weeks, when subcutaneously-grown tumors reached 10 mm in diameter, they were harvested and cut into small fragments. A 5-mm skin incision was made on the right thigh of nude mice. The biceps femoris was split and a 3–4 mm^3 single tumor fragment was implanted orthotopically into the space to establish the USTS PDOX model [29]. The wound was closed with a 6–0 nylon suture (UNIFY, AD Surgical, Sunnyvale, CA).

2.4. Treatment study design in the USTS PDOX model

The USTS PDOX mouse models were randomized into 7 groups of 8 mice each and treated with following drugs for 2 weeks (Fig. 1B): G1, untreated control; G2, OLA (60 mg/kg, intraperitoneal injection [i.p.], twice a week) alone; G3, DOX (3 mg/kg, i.p., once a week) alone; G4, DOX + IFO (30 mg/kg, i.p., three consecutive days in a week); G5, OLA + DOX; G6 OLA + IFO; G7, OLA + DOX + IFO. Treatment started when all tumors reached 100 mm^3. Tumor length, width and mouse body weight were measured twice per week. Tumor volume was calculated with the following formula: Tumor volume (mm^3) = length (mm) × width (mm) × width (mm) × 1/2. Data are presented as mean ± standard error of the mean (SEM).

2.5. Histological analysis

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was performed according to standard protocol. Ki-67 immunohistochemical staining with anti-Ki-67 antibody (Abcam Ltd., Cambridge, MA) in combination with diamino-benzidine (DAB, Dako Japan Inc., Kyoto, Japan), staining and hematoxylin counterstaining was performed according to manufacturer's protocols. The Ki-67 labeling index, the percentage of tumor cell nuclei with positive immunostaining above the background level, was calculated semi-quantitatively [31].

2.6. Statistical analysis

All statistical analyses were performed with statistical software EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3. 4. 1.). It is a modified version of R commander (version 2. 4–0) including statistical functions for biostatistics. A normal distribution was assessed with the Shapiro-Wilk test. Bartlett's test was used to verify the homogeneity of variances across groups. One-way ANOVA with Tukey HSD for post-hoc analysis was used for the parametric test for inter-group comparison. Kruskal-Wallis with Steel-Dwass for post-hoc analysis was used for the non-parametric test for inter-group comparison. The paired *t*-test was used for the parametric test to compare the means between two related groups. All *p*-values were two sided and *p*-values of 0.05 or less were considered statistically significant.

3. Results

3.1. Efficacy of chemotherapy on USTS PDOX

OLA alone and DOX alone did not significantly inhibit USTS PDOX growth compared with control ($p = 0.85$ and $p = 0.1$, respectively). However, DOX + IFO ($p < 0.001$), OLA + DOX ($p < 0.001$), OLA + IFO ($p < 0.001$), and OLA + DOX + IFO ($p < 0.001$) significantly inhibited tumor growth compared with the control. Only OLA + DOX + IFO had a significant increased efficacy compared to DOX alone ($p = 0.015$) (Fig. 2A and B, 3). A waterfall plot indicating the change in tumor volumes for the individual tumors is presented in Fig. 2B, which showed the tumor volume at day 14 relative to the initial tumor volume for each mouse.

3.2. Histology of the USTS PDOX

The tumor tissue of the control group mainly comprised viable highly-dense spindle-shaped tumor cells (Fig. 4A). Tumors treated with OLA (Fig. 4B) and DOX (Fig. 4C) alone comprised spindle-shaped viable cells, but the cancer-cell density was lower than the control. Wide degenerative scar change in the stroma and tumor necrosis with more cancer cells with karyorrhexis, swollen nuclei, or vacuolar degeneration were detected in the tumors treated with DOX + IFO, OLA + DOX, OLA + IFO, and OLA + DOX + IFO (Fig. 4D–G). The cancer-cell density was lowest and the degenerative scar change in the stroma was widest in PDOX tumors treated with OLA + DOX + IFO (Fig. 4G). The strong efficacy of the OLA + DOX + IFO combination on the USTS PDOX tumor was thus also shown histologically (Fig. 4).

3.3. Ki-67 immunohistochemical staining

In order to evaluate the proliferative capacity of cancer cells after treatment, immunohistochemcial staining with the Ki-67 proliferation marker, which is present during all active phases of the cell cycle and is absent in resting cells, was performed on tumor sections. We found that tumors treated with DOX + IFO (mean Ki-67 labeling index, 10.8%, $p = 0.02$), OLA + DOX (8.1%, $p = 0.04$), and OLA + DOX + IFO

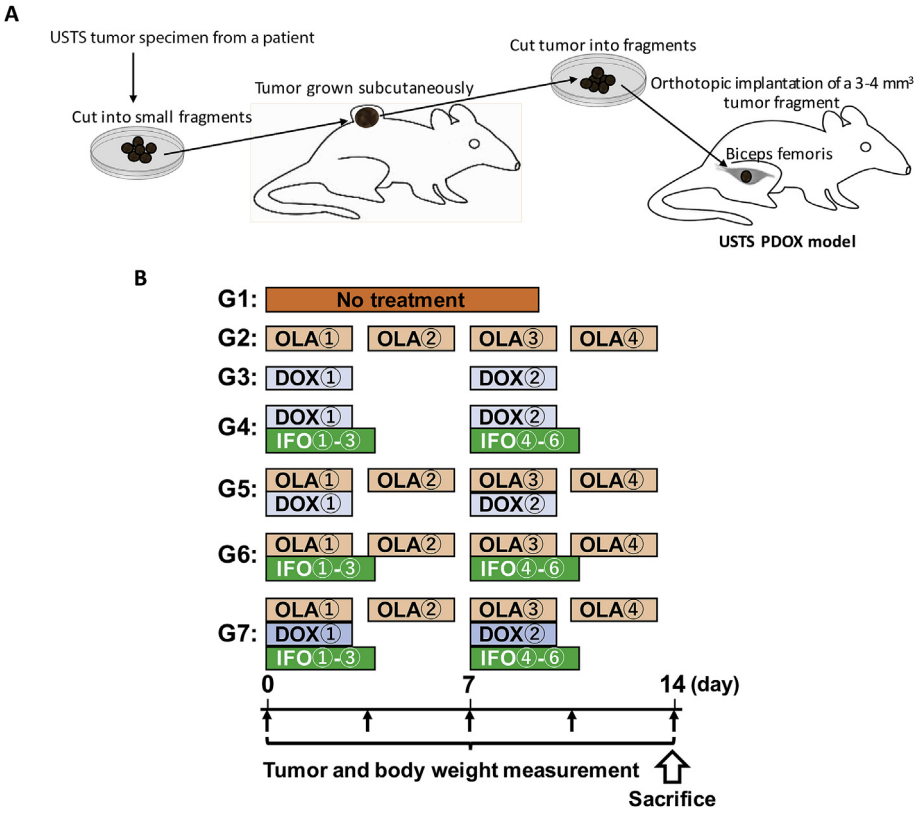


Fig. 1. (A) A schematic illustration of SOI for establishment of USTS PDOX model (modified after [48]). (B) Treatment regime for USTS PDOX model.

(8.6%, p = 0.01) had a significantly lower Ki-67 labeling index compared to the untreated control (39.3%). There was no significant difference in the Ki-67 labeling index of tumors treated with OLA (20.7%, p = 0.3), DOX (21.4%, p = 0.3), and OLA + IFO (16.8%, p = 0.1) compared with the control (Fig. 5).

3.4. Effect of treatment on body weight

Mouse body weight was measured pre-treatment and post-treatment. Final body weight of mice in control ($p < 0.001$), OLA ($p = 0.025$), and OLA + IFO ($p < 0.001$) increased compared with initial body weight. There was no significant difference in body weight among the other groups (Fig. 6). There were no observable other side effects or animal deaths in any group.

4. Discussion

Several combination therapies have been in the clinical trials for the

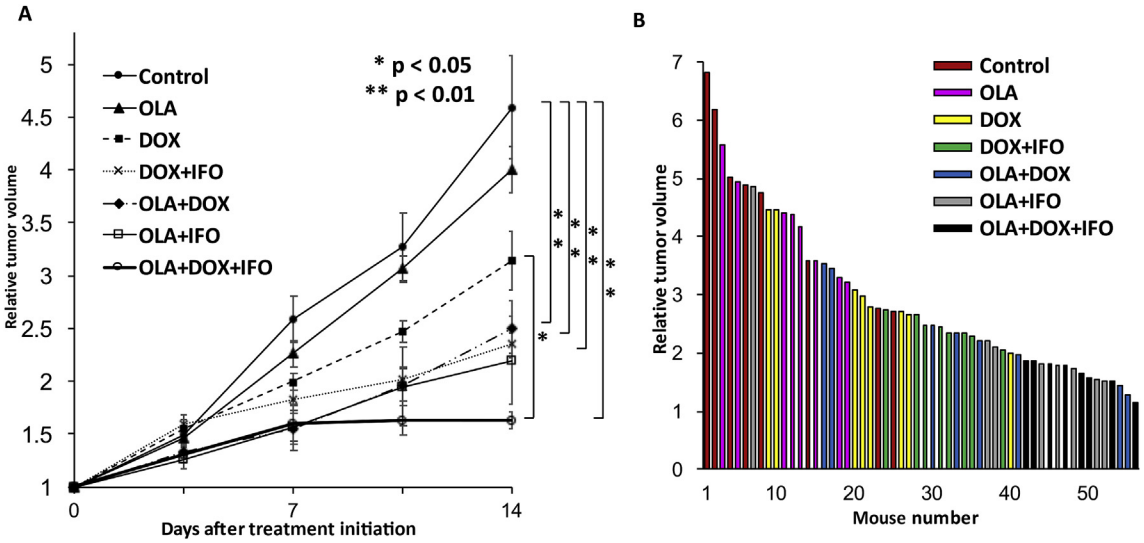


Fig. 2. (A) Quantitative efficacy of chemotherapy on the USTS PDOX model. Line graphs indicate relative tumor volume (tumor volume at each time point/tumor volume at onset of treatment) at each time point after the onset of treatment. Statistical analysis was performed with the one-way ANOVA with Tukey HSD for post-hoc analysis. N = 8 mice/group. *$p < 0.05$; **$p < 0.01$. Error bars: ± SEM. (B) Waterfall plot of tumor volume at day 14 relative to the initial tumor volume for each mouse.

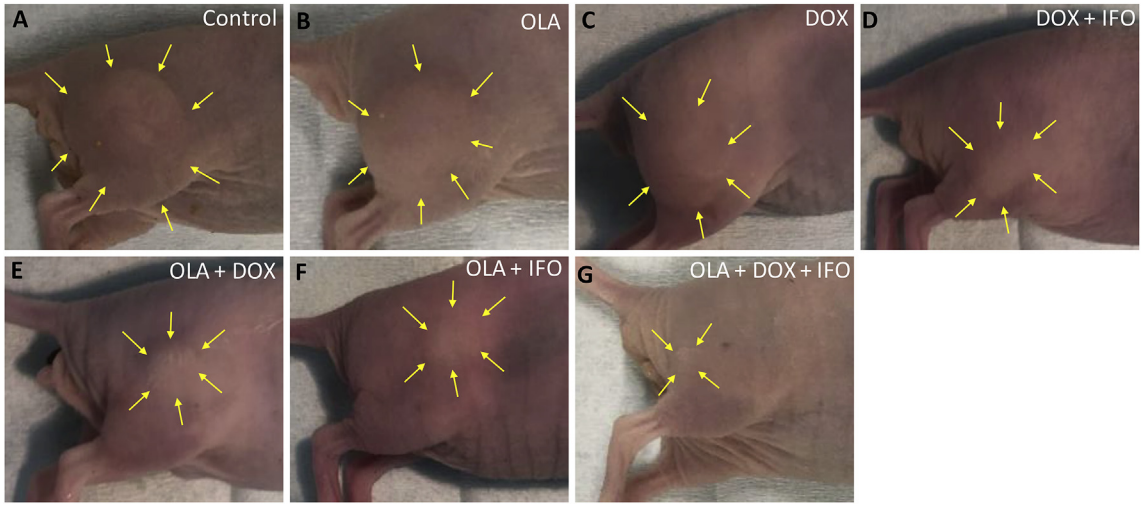


Fig. 3. Photographs of representative PDOX mouse models from each treatment group at day 14. Arrows indicate the margins of the tumors. (A) Untreated control. (B) OLA treated. (C) DOX treated. (D) DOX + IFO treated. (E) OLA + DOX treated. (F) OLA + IFO treated. (G) OLA + DOX + IFO treated.

treatment for STS such as GEM + sirolimus [12], IFO + sorafenib [32], GEM + DOX plus bevacizumab [33,34]. However, so far, no effective treatment regimens for USTS has been developed. In the present study, we found that the OLA + DOX and IFO combination can arrest the USTS PDOX model. This is the first study which shows that OLA + DOX and IFO combination is active in USTS, in this case a tumor resistant to both OLA and DOX alone.

OLA is first-in-class–selective PDGFRα inhibitor [35]. OLA was well tolerated and showed antitumor activity in patients with advanced solid tumors [36,37]. OLA was shown to reduce proliferation and progression of several cancer cell lines including sarcomas [20,38]. A recent Phase II study showed a prolonged survival in advanced STS patients when OLA was combined with DOX [22]. Jones et al. [39] characterized the exposure–response relationship of OLA for progression-free survival, overall survival, and safety when combined with DOX in patients with advanced STS and found that OLA is efficacious and less toxic. OLA plus DOX had an acceptable safety profile in Japanese patients with advanced STS [40]. DOX and IFO has been used for the treatment of the patients with STS for more than 30 years and is their most common chemotherapy regimen [1,13,14]. In the present study, adding OLA to the DOX and IFO combination could arrest the tumor growth in USTS PDOX. As far as we are aware, this is the first in vivo study demonstrating the efficacy of the combination of OLA and DOX plus IFO on STS. Further studies are needed to confirm the tolerability of this combination in patients.

There is an ongoing Phase I clinical trial testing the efficacy of OLA together with DOX and IFO in the treatment of patients with advanced STS (NCT03283696) [41]. OLA alone or in combination with DOX was used to block the growth of preclinical mouse models of PDGFRα-expressing pediatric osteosarcoma and malignant rhabdoid tumor models [42]. A combination of OLA with DOX inhibited tumor growth compared with DOX alone in xenograft models of human osteosarcoma [43]. In a Phase II clinical trial, OLA had an acceptable safety profile in patients with pretreated GIST [44]. Patients with PDGFRα-mutant GIST treated with OLA had prolonged disease control, but no efficacy was found in patients with GIST without PDGFRα mutations [44]. However, Gerber et al. [45] found no efficacy of OLA combined with paclitaxel/carboplatin or paclitaxel/carboplatin alone in previously untreated advanced non-small cell lung cancer (NSCLC). Similarly, in a randomized, open label Phase II study, OLA together with liposomal DOX was compared with liposomal DOX alone in advanced ovarian cancer patients, but did not result in significant prolongation of progression-free survival (PFS) or overall survival in platinum-refractory ovarian cancer [46]. A recent Phase II study examined the efficacy and safety of a combination of OLA, mitoxantrone and prednisone (M/P) compared to M/P alone in patients with metastatic castration-resistant prostate

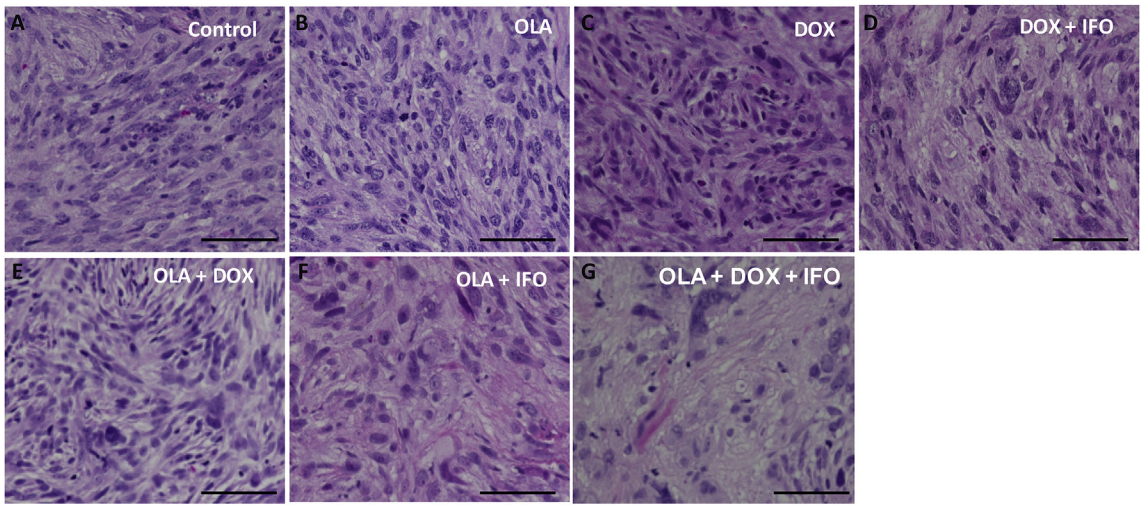


Fig. 4. Tumor histology. (A) Untreated control. (B) OLA. (C) DOX. (D) DOX + IFO. (E) OLA + DOX. (F) OLA + IFO. (G) OLA + DOX + IFO. Scale bars: 100 μm.

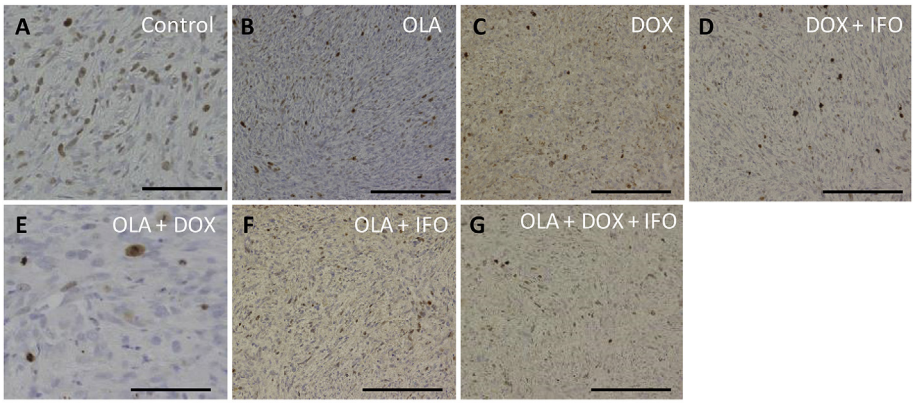
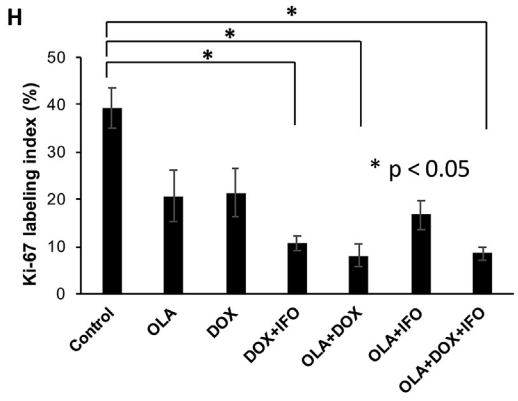


Fig. 5. Ki-67 immunochemical histology. (A) Untreated control. (B) OLA. (C) DOX. (D) DOX + IFO. (E) OLA + DOX. (F) OLA + IFO. (G) OLA + DOX + IFO. Scale bars: 100 μm. (H) Ki-67 labeling index. Bar graphs show the percentage of cancer cell nuclei with positive immunostaining. Statistical analysis was performed with the Kruskal-Wallis with Steel-Dwass for post-hoc analysis. N = 6 fields/group. *$p < 0.05$.



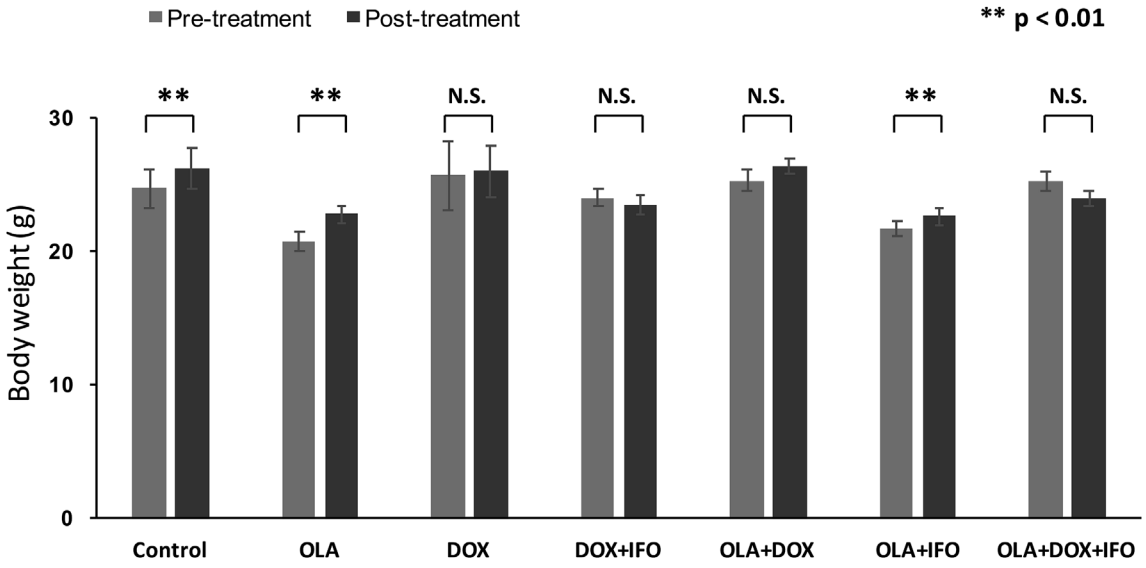


Fig. 6. Mouse body weight. Bar graphs show mouse body weight in each treatment or control group at pre- and post-treatment time. Statistical analysis was performed with the paired-t test. **$p < 0.01$, N.S., not significant.

cancer [47]. They found that OLA was safe but did not improve the efficacy of M/P chemotherapy [47]. Further studies are needed to confirm PDGFRα mutations and change in the phospho-PDGFRα positive rate after OLA treatment in the USTS tumor.

In conclusion, this study uniquely demonstrates the power of the PDOX model to identify novel effective therapy using the OLA plus DOX and IFO combination for USTS. The data presented here suggest that combination of OLA plus DOX and IFO could be a promising novel therapy for USTS.

Conflicts of interest

TH, KM, NS, HO, ZZ, SR, NY, KH, HK, SM, KI and RMH are or were unsalaried associates of AntiCancer Inc. AntiCancer Inc. uses PDOX models for contract research. The authors declare that they have no competing interests.

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