

High Efficacy of Pazopanib on an Undifferentiated Spindle-Cell Sarcoma Resistant to First-Line Therapy Is Identified With a Patient-Derived Orthotopic Xenograft (PDOX) Nude Mouse Model

Kentaro Igarashi,^{1,2,3} Kei Kawaguchi,^{1,2} Takashi Murakami,^{1,2} Tasuku Kiyuna,^{1,2} Kentaro Miyake,^{1,2} Arun S. Singh,⁴ Scott D. Nelson,⁵ Sarah M. Dry,⁵ Yunfeng Li,⁵ Norio Yamamoto,³ Katsuhiko Hayashi,³ Hiroaki Kimura,³ Shinji Miwa,³ Hiroyuki Tsuchiya,³ Fritz C. Eilber,^{6*} and Robert M. Hoffman^{1,2**}

¹AntiCancer, Inc., San Diego, California

²Department of Surgery, University of California, San Diego, California

³Department of Orthopedic Surgery, Kanazawa University, Kanazawa, Japan

⁴Division of Hematology-Oncology, University of California, Los Angeles, California

⁵Department of Pathology, University of California, Los Angeles, California

⁶Division of Surgical Oncology, University of California, Los Angeles, California

ABSTRACT

Undifferentiated spindle-cell sarcoma (USCS) is a recalcitrant cancer. Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI). In the present study, we evaluated the efficacy of standard first-line chemotherapy of doxorubicin (DOX), gemcitabine (GEM) combined with docetaxel (DOC), compared to pazopanib (PAZ), a multi-targeting tyrosine-kinase inhibitor, in an USCS PDOX model. A high-grade USCS from a striated muscle of the patients was grown orthotopically in the right biceps femoris muscle of nude mice to establish the PDOX model. The PDOX models were randomized into the following groups when tumor volume reached 100 mm³: G1, control without treatment; G2, DOX (3 mg/kg, intraperitoneal (i.p.) injection, weekly, for 2 weeks); G3, GEM (100 mg/kg, i.p., weekly, for 2 weeks) combined with DOC (20 mg/kg, i.p., once); G4, PAZ (100 mg/kg, p.o., daily, for 14 days). All treatments except DOX significantly inhibited tumor growth compared to untreated control on day 14 after treatment initiation. Tumor sizes were as follows: control (G1): 332.0 ± 58.7 mm³; DOX (G2): 316.9 ± 55.9 mm³, *P* = 0.605; GEM + DOC (G3): 228.9 ± 39.8 mm³, *P* = 0.001; PAZ (G4): 173.8 ± 23.3 mm³, *P* < 0.0001. PAZ showed significantly more efficacy compared to other therapies evaluated: DOX (*P* < 0.0001), GEM + DOC (*P* = 0.006). There were no animal deaths in any group and body weight of treated mice was not significantly different in each group. The present results demonstrate that the PDOX model of USCS can identify a promising novel agent with significantly greater efficacy than first-line therapy for this recalcitrant disease. *J. Cell. Biochem.* 118: 2739–2743, 2017. © 2017 Wiley Periodicals, Inc.

KEY WORDS: SPINDLE-CELL SARCOMA; PDOX; NUDE MICE; PAZOPANIB; DOXORUBICIN; GEMCITABINE; DOCETAXEL; INDIVIDUALIZED THERAPY; PRECISION MEDICINE; EFFICACY; RESISTANCE

Spindle-cell sarcoma is a recalcitrant cancer which has predominant spindle-shaped cells. Spindle-cell carcinoma can originate in nerve sheath, layers of connective tissue such as under the skin, in muscles, and other organs. Spindle-cell sarcomas

are fibromyxoid with characteristics of both mesenchymal and neuroendocrine differentiation [Shaikh et al., 2014]. A balanced translocation between chromosomes 7 and 16 has been identified in a case of spindle-cell carcinoma [Reid et al., 2003].

*Correspondence to: Fritz C. Eilber, M.D., Professor of Surgery, Professor of Molecular & Medical Pharmacology UCLA, Division of Surgical Oncology, 10833 LeConte Avenue, Rm 54-140 CHS, Los Angeles, CA 90095-1782. E-mail: fceilber@mednet.ucla.edu

**Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111. E-mail: all@anticancer.com

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Soft-tissue sarcomas (STS) are heterogeneous, which require development of individualized therapeutic strategies for patients, instead of standard chemotherapy for all patients [Radaelli et al., 2014].

In order to pursue the goal of precision individualized precision medicine, our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI). PDOX models thus far developed are pancreatic [Fu et al., 1992; Hiroshima et al., 2014a,b, 2015a], breast [Fu et al., 1993], ovarian [Fu and Hoffman, 1993], lung [Wang et al., 1992], cervical [Hiroshima et al., 2015b; Murakami et al., 2017a], colon [Fu et al., 1991; Metildi et al., 2014; Hiroshima et al., 2014c], and stomach cancers [Furukawa et al., 1993], sarcoma [Hiroshima et al., 2015c,d; Kiyuna et al., 2016; Murakami et al., 2016a,b], and melanoma [Yamamoto et al., 2016; Kawaguchi et al., 2016a,b]. PDOX models of sarcoma that we have developed include undifferentiated pleomorphic sarcoma (UPS) [Murakami et al., 2016a; Kiyuna et al., 2017], follicular dendritic cell sarcoma (FDCS) [Kiyuna et al., 2016], rhabdomyosarcoma (RMS) [Igarashi et al., 2017], Ewing's sarcoma [Murakami et al., 2016b], and osteosarcoma [Murakami et al., 2017b]. The present manuscript describes a PDOX model of undifferentiated spindle-cell sarcoma (USCS).

The combination of gemcitabine (GEM) and docetaxel (DOC) has shown efficacy on STS [Hensley et al., 2002; Leu et al., 2004; Bay et al., 2006; Maki et al., 2007].

Pazopanib (PAZ) is a synthetic indazolympyrimidine that is an inhibitor of multiple tyrosine kinases [Schoffski, 2012]. A phase III clinical trial for metastatic STS has been carried out to evaluate the efficacy of PAZ. A significant 3-month advantage in progression-free survival (PFS) was achieved by PAZ [van der Graaf et al., 2012].

In the present study, we compared the efficacy of PAZ against first-line therapy with doxorubicin (DOX) and the combination of GEM and DOC in a PDOX model of USCS.

MATERIALS AND METHODS

MICE

Athymic nu/nu nude mice (AntiCancer Inc., San Diego, CA), 4–6 weeks old, were used in this study. Animals were housed in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under standard conditions of 12-h light/dark cycles. The animals were fed an autoclaved laboratory rodent diet. All animal studies were conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. In order to minimize any suffering of the animals the use of anesthesia and analgesics were used for all surgical experiments. Animals were anesthetized by subcutaneous injection of a 0.02 ml solution of 20 mg/kg ketamine, 15.2 mg/kg xylazine, and 0.48 mg/kg acepromazine maleate.

PATIENT-DERIVED TUMOR

A 56-year-old male diagnosed with USCS in his right shoulder underwent surgical resection at Department of Surgery, University of California, Los Angeles (UCLA). 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).

SURGICAL ORTHOTOPIC IMPLANTATION (SOI) FOR ESTABLISHMENT OF PDOX MODEL

A fresh sample of the tumor of the patient was obtained and transported immediately to the laboratory at AntiCancer, Inc., on wet ice. The sample was cut into 5-mm fragments and implanted subcutaneously in nude mice. The subcutaneous xenograft was grown and established 3 weeks later. The grown tumors were cut into small fragments (3–4 mm). After nude mice was anesthetized, a 5 mm skin incision was made on the right high thigh, the biceps femoris was split and a single tumor fragment was implanted orthotopically into the space to establish the PDOX model. The wound was closed with a 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ).

TREATMENT STUDY DESIGN IN THE PDOX MODEL OF USCS

PDOX mouse models were randomized into four groups of eight mice each: G1, control without treatment; G2, (DOX) (3 mg/kg, intraperitoneal (i.p.) injection, qw × 2); G3, (GEM) (100 mg/kg, i.p., qw × 2) combined with (DOC) (20 mg/kg, i.p., once); G4, (PAZ) (100 mg/kg, p.o., qd × 14). Tumor length, width, and mouse body weights were measured twice in a week. Tumor volume was calculated by following formula: tumor volume (mm³) = length (mm) × width (mm) × width (mm) × 1/2. (Fig. 1).

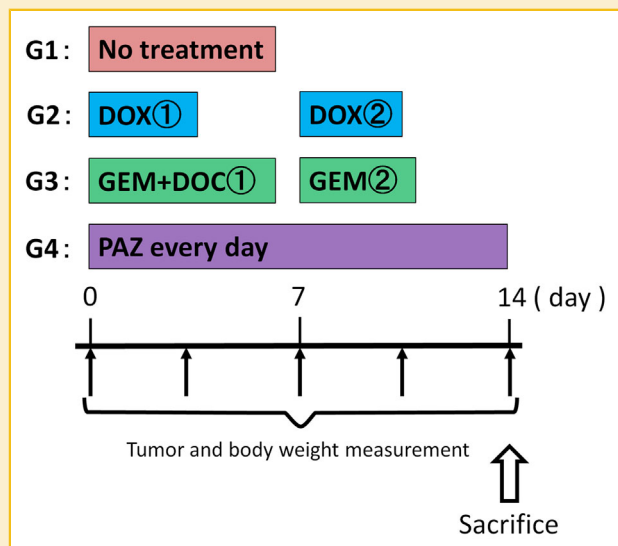


Fig. 1. Treatment schema. Undifferentiated spindle cell sarcoma (USCS) was grown orthotopically in the right biceps femoris muscle of nude mice and allowed to form tumors. Mice were treated with the following: DOX: 3 mg/kg/week i.p. for 2 weeks; GEM: 100 mg/kg, i.p., weekly for 2 weeks combined with DOC: 20 mg/kg, i.p., once; PAZ: 100 mg/kg, p.o., daily for 14 days. Tumor volume was measured at the indicated time points after the onset of treatment. N = 8 mice/group.

HISTOLOGICAL ANALYSIS

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (3 μm) were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was performed according to standard protocol. Histological examination was performed with a BHS system microscope. Images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada).

STATISTICAL ANALYSIS

SPSS statistics version 21.0 was used for all statistical analyses (IBM, New York City, NY). Significant differences for continuous variables were determined using the Student's *t*-test. Both line graphs and bar graphs expressed mean values and error bars show standard deviation (SD). A probability value of *P* was calculated between control group and each treatment group. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

EFFECT OF PAZ, DOX, GEM + DOC ON THE USCS PDOX MOUSE MODEL

The USCS PDOX was resistant to DOX and partially sensitive to GEM-DOC and highly sensitive to PAZ. Tumor sizes were as follows: control (G1): $332.0 \pm 58.7 \text{ mm}^3$; DOX (G2): $316.9 \pm 55.9 \text{ mm}^3$, $P = 0.605$; GEM + DOC (G3): $228.9 \pm 39.8 \text{ mm}^3$, $P = 0.001$; PAZ (G4): $173.8 \pm 23.3 \text{ mm}^3$, $P < 0.0001$. PAZ showed significantly more efficacy compared to the other therapies evaluated: DOX ($P < 0.0001$), GEM + DOC ($P = 0.006$) (Fig. 2). There were no animal deaths in any group. Body weight of treated mice were not significantly different in each group (Fig. 3).

HISTOLOGY

High power photomicrographs of the original USCS patient tumor displayed solid sheets of tumor cells characterized by spindle cells with hyperchromatic, enlarged tapering nuclei. Numerous mitotic

figures, including atypical forms are present. High power photomicrographs of orthotopically implanted USCS had nearly identical features including spindle-shaped cells with hyperchromatic, enlarged tapering nuclei. Numerous mitotic figures, including atypical forms are also present (Fig. 4).

Tumors treated with DOX were comprised of spindle-shaped viable cells without apparent necrosis or inflammatory changes. Tumors treated with GEM + DOC showed changes in cancer-cell shapes with slight necrosis. PAZ-treated tumors showed more extensive tumor necrosis (Fig. 4).

In the present study, a novel PDOX model of USCS was established. The USCS PDOX was resistant to DOX, a first-line therapy. The combination of GEM and DOX was only partially effective. However, the experimental agent PAZ was significantly more effective than current first-line therapy DOX and GEM-DOC. The novel spindle-cell sarcoma PDOX has the potential to individualize and improve therapy for the patient and for drug discovery of this recalcitrant disease.

The PDOX models have been shown to identify effective therapy for multiple types of sarcomas:

In a PDOX model of high-grade UPS from a striated muscle, the tumor was eradicated by treatment with tumor-targeting *Salmonella typhimurium* A1-R followed by DOX [Murakami et al., 2016a].

A PDOX models of FDSC was resistant to both DOX and NVP-BEZ235, dactolisib (BEZ) an experimental agent which is a dual pan-phosphoinositide 3-kinase-mammalian target of rapamycin inhibitor. However, in contrast to DOX and BEZ, the FDSC PDOX was sensitive to *S. typhimurium* A1-R. The combination of *S. typhimurium* A1-R and either DOX or BEZ did not increase the antitumor efficacy of *S. typhimurium* A1-R, indicating that DOX and BEZ were not active in this PDOX model [Kiyuna et al., 2016].

A PDOX model was established in the right chest wall of nude mice from a Ewing's sarcoma with cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) loss and FUS-ERG fusion. Tumor growth was significantly suppressed by palbociclib and linsitinib compared to untreated controls. In contrast, DOX did not inhibit tumor growth at any time point, which is consistent with the failure of DOX to control tumor growth in the patient [Murakami et al., 2016b].

These studies demonstrate the potential of the PDOX models for individualized precision treatment of the recalcitrant heterogeneous group of sarcomas. The present study suggests that the spindle-cell sarcoma patient could benefit from the novel agent PAZ.

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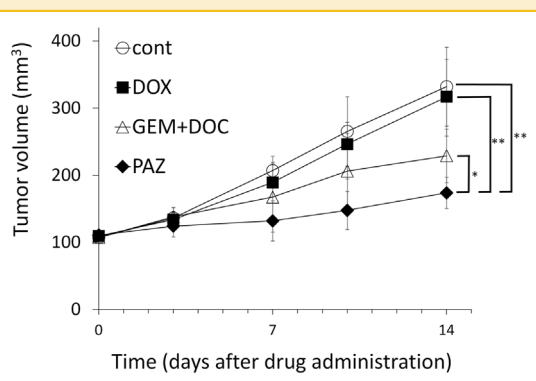


Fig. 2. Efficacy of DOX, or GEM combined with DOC, or PAZ on the USCS PDOX. Line graphs show tumor volume relative to the beginning of treatment. * $P < 0.01$, ** $P < 0.001$.

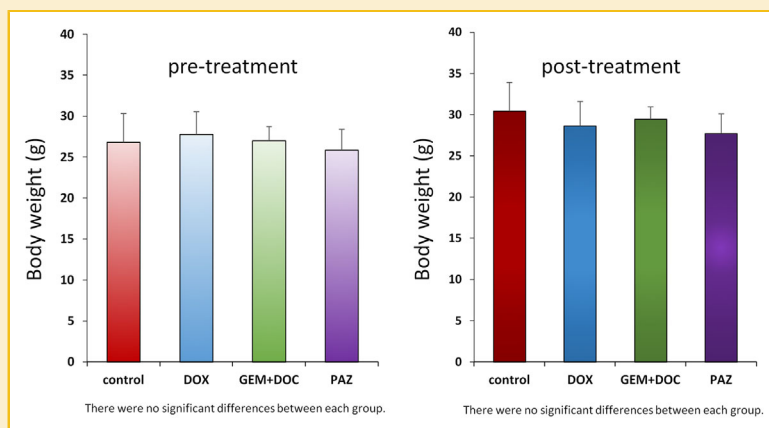


Fig. 3. Body weight response to treatment. Bar graph shows body weight in each group at pre-treatment and 2 weeks after drug administration. n.s., no significant difference.

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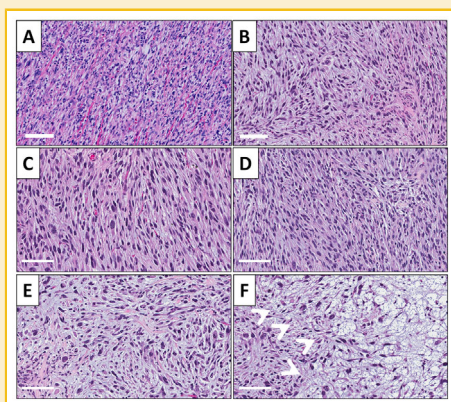


Fig. 4. Histology of the original patient tumor and the untreated control PDOX tumor. Hematoxylin and eosin (H&E)-stained section of the original patient tumor (A); untreated PDOX tumor (low magnification) (B); Untreated PDOX tumor (high magnification) (C); PDOX tumor treated with DOX (high magnification) (D); PDOX tumor treated with GEM + DOC (high magnification) (E); PDOX tumor treated with PAZ (high magnification) (F). Scale bars: 100 μm (A and B). Scale bars: 80 μm (C–F). Arrowheads in (F) indicate necrotic area of tumor.

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