UCLA

UCLA Previously Published Works

Title

Growth of doxorubicin-resistant undifferentiated spindle-cell sarcoma PDOX is arrested by metabolic targeting with recombinant methioninase.

Permalink

<https://escholarship.org/uc/item/6wh637kb>

Journal

Journal of cellular biochemistry, 119(4)

ISSN

0730-2312

Authors

Igarashi, Kentaro
Li, Shukuan
Han, Qinghong
et al.

Publication Date

2018-04-01

DOI

10.1002/jcb.26527

Peer reviewed

Learn new strategies for:

Cell-based
immunotherapies:

T-Cell CARS

Webinar Details:

9:00 AM EST(US): Dec 12

7:00 AM CST(China): Dec 13

[Register Here](#)

Article

Growth of doxorubicin-resistant undifferentiated spindle-cell sarcoma PDOX is arrested metabolic targeting with recombinant methioninase[†]

Running title: Recombinant methioninase arrests a doxorubicin-resistant spindle-cell sarcoma

Kentaro Igarashi^{1,2,3}, Shukuan Li¹, Qinghong Han¹, Yuying Tan¹, Kei Kawaguchi^{1,2}, Takashi Murakami^{1,2}, Tasuku Kiyuna^{1,2}, Kentaro Miyake^{1,2}, Yunfeng Li⁴, Scott D. Nelson⁴, Sarah M. Dry⁴, Arun S. Singh⁵, Irmina A. Elliott⁶, Tara A. Russell⁶, Mark A. Eckardt⁷, Norio Yamamoto³, Katsuhiko Hayashi³, Hiroaki Kimura³, Shinji Miwa³, Hiroyuki Tsuchiya³, Fritz C. Eilber⁶ and Robert M. Hoffman^{1,2}

¹ AntiCancer, Inc., San Diego, California

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

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

⁴ Dept. of Pathology, University of California, Los Angeles, CA

⁵ Division of Hematology-Oncology, University of California, Los Angeles, CA

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

⁷ Department of Surgery, Yale School of Medicine, New Haven, Connecticut

Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer, Inc., 7917 Ostrow St., San Diego, CA 92111, Tel: 858-654-2555, Fax: 858-268-4175, Em: all@anticancer.com -or- 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, Tel: 310-825-7086, Fax: 310-825-7575, Em: fceilber@mednet.ucla.edu -or- Hiroyuki Tsuchiya, MD, Ph.D., Dept. of Orthopaedic Surgery, Graduate School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan, Tel: +81 762652374, Fax: +81 762344261, e-mail: tsuchi@med.kanazawa-u.ac.jp

[†]This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcb.26527]

Received 21 July 2017; Revised 6 September 2017; Accepted 13 November 2017

Journal of Cellular Biochemistry

This article is protected by copyright. All rights reserved

DOI 10.1002/jcb.26527

Abstract

Undifferentiated spindle-cell sarcoma (USCS) is a recalcitrant cancer in need of individualized therapy. A high-grade USCS from a striated muscle of a patient was grown orthotopically in the right biceps femoris muscle of nude mice to establish a patient-derived orthotopic xenograft (PDOX) model. In a previous 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. In the present study, animal-bearing the USCS PDOX tumors were randomized into the following groups when tumor volume reached 100 mm³: G1, untreated control without treatment; G2, DOX (3 mg/kg, intraperitoneal (i.p.) injection, weekly, for 2 weeks); G3, L-methionine α -deamino- γ -mercaptomethane lyase (recombinant methioninase [rMETase]) (100 unit/mouse, i.p., daily, for 2 weeks). Tumor sizes and body weight were measured with calipers and digital balance twice a week and methionine level of supernatants derived from sonicated tumors were measured. rMETase inhibited tumor growth compared to untreated controls and the DOX treated group on day 14 after initiation of treatment: control (G1): 347.6 ± 88.2 mm³; DOX (G2): 329.5 ± 78.8 mm³, $p=0.670$; rMETase (G3): 162.6 ± 51.2 mm³, $p=0.0003$. The mouse body weight of the treated mice was not significantly different from the untreated controls. Tumor L-methionine levels were reduced after the rMETase-treatment compared to untreated control and pre-rMETase treatment. We previously reported efficacy of rMETase against Ewing's sarcoma in a PDOX model. These studies suggest clinical development of rMETase, especially in recalcitrant cancers such as sarcoma. This article is protected by copyright. All rights reserved

Key words: undifferentiated spindle-cell sarcoma, doxorubicin, resistant, patient-derived orthotopic xenograft, PDOX, recombinant methioninase

Introduction

Undifferentiated spindle-cell sarcoma (USCS) is a recalcitrant cancer sarcoma which has predominant spindle-shaped cells which can originate in nerve sheaths, layers of connective tissue such as that under the skin, in muscles, and other organs. Spindle cell sarcomas have been characterized as 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; Igarashi et al., 2017a]. 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 a PDOX model. In a previous 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. The USCS first line therapy was resistant to DOX but significantly inhibited by the combination of gemcitabine (GEM) and docetaxel (DOC) and pazopanib (PAZ). PAZ showed significantly more efficacy compared to GEM+DOC. These results demonstrated that the PDOX model of USCS can identify a promising novel agent with significantly greater efficacy than first-line therapy for this recalcitrant disease [Igarashi et al., 2017a].

Methionine dependence is due to the overuse of methionine for aberrant transmethylation reactions in cancer. In order to exploit methionine dependence for therapy, our laboratory previously cloned L-methionine α -deamino- γ -mercaptomethane lyase (recombinant methioninase [rMETase]). We previously reported efficacy of rMETase against Ewing's sarcoma in a PDOX model [Murakami et al., 2017a].

The present report demonstrates the efficacy of recombinant methioninase on the PDOX model of USS compared to conventional DOX therapy.

Materials and Methods

Mice

Athymic *nu/nu* female 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-hour light/dark cycles. The animals were fed an autoclaved laboratory rodent diet. All animal studies were conducted with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principals and procedures outlined in the National Institutes 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. The response of animals during surgery was monitored to ensure adequate depth of anesthesia. The animals were observed on a daily basis and humanely sacrificed by CO₂ inhalation when they met the following humane endpoint criteria: severe tumor burden (more than 20 mm in diameter), prostration, significant body weight loss, difficulty breathing, rotational motion and body temperature drop [Igarashi et al., 2017a].

Patient-derived tumor

A 56-year-old male diagnosed with USCS at primary right shoulder previously underwent surgical resection at Department of Surgery, University of California, Los Angeles (UCLA). He 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 (IRB #10-001857)-approved protocol [Igarashi et al., 2017a].

Surgical orthotopic implantation (SOI) for establishment of PDOX model

A single USCS tumor fragment, previously grown subcutaneously in nude mice, was implanted orthotopically into the right thigh to establish a PDOX model. The wound was closed with 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ, USA) [Igarashi et al., 2017a].

Recombinant methioninase (rMETase) production

The pAC-1 rMETase high expression clone was used for rMETase production. The fermentation procedure for host E.coli cells and the purification protocol for rMETase were the same as previously described: rMETase was purified by 3 different steps using columns of DEAE Sepharose FF and Sephacryl S-200HR, and ActiClean Etox, which is designed for eliminating endotoxin [Tan et al., 1997].

Treatment study design

The USCS PDOX mouse models were randomized into 4 groups of 8 mice each: G1, untreated control without treatment; G2, DOX (3 mg/kg, intraperitoneal (i.p.) injection, weekly, for 2 weeks); G3, rMETase (100 unit/mouse in 250 μ l phosphate buffered saline [PBS], i.p., daily, for 2 weeks). Tumor length, width and mouse body weight were measured twice in a week. Tumor volume was calculated by following formula: Tumor volume (mm^3) = length (mm) $\times$ width (mm) $\times$ width (mm) $\times$ 1/2. Data was presented as mean $\pm$ SD.

Tumor protein level measurement

To standardize tumor L-methionine measurements, tumor protein levels were measured. Briefly, each PDOX tumor was placed in phosphate buffered saline (PBS) (1 ml). Tumors were sonicated on ice for 30 seconds and subsequently centrifuged at 12,000 rpm for 10 minutes.

Supernatants were diluted to the concentration ranging from 200 to 1500 µl/ml. Protein assay reagent (Bio-Rad, Hercules, CA) was prepared as a 4-fold dilution and added to each tube with the sonicated tumor supernatants, then absorbance of 595 nm was measured by the microprocessor controlled microplate reader (Sunrise™; TECAN, San Joes, CA, USA). Protein levels were calculated from the standard curve obtained by protein standard, bovine serum albumin (BSA) [Murakami et al., 2017a].

Tumor L-methionine level analysis at termination

Tumor supernatants obtained from the above-described sonication procedure were precipitated by acetonitrile. L-methionine levels were measured with an HPLC (Hitachi L-6200A Intelligent pump; Hitachi, Ltd., Tokyo, Japan) after derivatization of supernatants amino acids with the fluoraldehyde reagent OPA as described previously [Tan et al., 1997; Sun et al., 2005]. L-methionine levels were determined as nmol/ml with the HPLC procedure described above. Standardized L-methionine levels were calculated using the following formula: standardized L-methionine level (nmol/mg protein) = L-methionine level (nmol/ml) / protein level (mg protein/ml) [Murakami et al., 2017a].

Histological examination

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) [Murakami et al., 2017a].

Result and Discussion

rMETase inhibited tumor growth compared to untreated control and DOX-treated mice. At day 14 after treatment initiation, tumor volumes were as follows: control (G1): $347.6 \pm 88.2 \text{ mm}^3$; DOX (G2): $329.5 \pm 78.8 \text{ mm}^3$, $p=0.670$; rMETase (G3): $162.6 \pm 51.2 \text{ mm}^3$, $p=0.0003$ (Figures 1-3). We have previously published correlations of tumor volume to tumor weight [Katz et al., 2003; Hiroshima, et al. 2014a]. The body weight of the treated mice was not significantly different from the untreated control (Figure 4). Tumor L-methionine levels were significantly reduced after the rMETase-treatment compared to untreated control and pre-rMETase treatment (Figure 5).

Figure 5 shows methionine (MET) levels at 2 time-points: pre-and post-treatment for the untreated control and the recombinant methioninase (rMETase)-treated groups. In the untreated control, the tumor MET increased by the end of the treatment group. The larger untreated tumor probably required more methionine in order to increase in size, as tumors are highly methionine-dependent for growth [Hoffman, 2015]. However, this increase in MET was not statistically significant. In contrast, the rMETase-treated tumors at the end of the experiment had a statistically-significant decrease in MET, with respect to the control tumors at the beginning and end of the treatment period and from the rMETase-treated-tumors at the beginning of the experiment. This experiment shows, with statistical-significance, that the rMETase-treatment reduced tumor MET levels, which is the probable mechanism for the tumor growth inhibition observed in Figure 3.

DOX was chosen as a treatment control because DOX is first-line therapy of sarcoma [Murakami et al., 2016a] and we wished to learn if rMETase treatment would be an improvement over

first-line therapy for this recalcitrant disease. The other control was no treatment. Therefore, we could compare the efficacy of DOX and rMETase against an untreated control.

Histology

High power photomicrographs of the original patient tumor showed cancer cells characterized by spindle shaped with hyperchromatic, enlarged tapering nuclei. Mitotic figures and atypical forms are present (Figure 6A). A high power view of the orthotopically implanted tumor showed similar features including spindle-shaped cells with hyperchromatic, enlarged tapering nuclei. Numerous mitotic figures, including atypical forms are also present (Figure 6B). Tumors treated with DOX were comprised of spindle shaped viable cells without apparent necrosis or inflammatory changes (Figure 6C). Tumors treated with rMETase showed changes in tumor cell shapes with apparent tumor necrosis (Figure 6D).

The first hint that methionine metabolism is perturbed in cancer came almost 60 years ago when Sugimura et al. observed that tumor growth in rats was slowed a defined diet depleted in methionine [Sugimura et al., 1959]. Approximately 45 years ago, it was observed that L5178Y mouse leukemia cells in culture required abnormally high levels of methionine to proliferate [Chello et al., 1973]. Subsequently, most cancer cell lines were found to be methionine dependent [Mecham et al., 1983; Tan et al., 2010; Hoffman 2017].

Multiple lines of evidence indicated that methionine-dependent cancer cells synthesize large amounts of methionine endogenously through the reaction catalyzed by N5-methyltetrahydropteroyl-L-glutamate: l-homocysteine S-methyltransferase (EC 2.1.1.13). [Hoffman and Erbe 1976; Hoffman 2015, 2017].

However, the amount of endogenous methionine, the level of free methionine and S-adenosylmethionine (AdoMET), the universal methyl donor, were very low in

methionine-dependent cancer cells in methionine limiting conditions, despite normal amounts of methionine synthesized [Coalson et al., 1982; Stern et al., 1983].

Cancer cells have enhanced overall rates of transmethylation compared with normal cells. The enhanced transmethylation rates may be the basis of the methionine dependence of cancer cells which explains the low levels of free methionine and the low AdoMET/AdoHCY ratio in cancer cells under methionine deprivation, despite high rates of methionine synthesis [Stern et al., 1984]. The elevated methionine use in cancer cells has been termed the “Hoffman effect.” [Murakami et al., 2017a].

Growth arrest of methionine-dependent cancer cells under conditions of methionine restriction arrest was in the S/G₂ phases of the cell cycle [Hoffman and Jacobsen 1980; Yano et al., 2014].

Methionine independent revertants

Rare cells from methionine-dependent cancer cell lines regained the normal ability to grow under methionine restriction. These lines were termed methionine-independent revertants [Hoffman et al., 1978]. Methionine-dependent revertants also had much lower basal transmethylation rates than parental methionine-dependent cell lines [Judde et al., 1989]. These results further suggested that methionine dependence is due to an increase in the rate of transmethylation reactions. We then demonstrated that methionine-independent revertants cells simultaneously reverted for characteristics associated with cancer and became less malignant. Thus, the methionine independent revertants become more normal-like indicating further a relationship between altered methionine metabolism and oncogenic transformation [Hoffman et al., 1979].

Toward the goal of precision personalized oncology, our laboratory pioneered the

Accepted Article

patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI), including pancreatic [Hiroshima et al., 2014b,c, 2015a; Fu et al., 1992; Kawaguchi et al., 2017a], breast [Fu et al., 1993a], ovarian [Fu and Hoffman 1993], lung [Wang et al., 1992], cervical [Hiroshima et al., 2015b; Murakami et al., 2017b], colon [Hiroshima et al., 2014d; Fu et al., 1991; Metildi et al., 2014], stomach [Furukawa et al., 1993], sarcoma [Murakami et al., 2016a, b, 2017c; Igarashi et al., 2017ab, Hiroshima et al., 2015c, Kawaguchi et al., 2017b; Kiyuna et al., 2016] and melanoma [Yamamoto et al., 2016; 38-42].

The present report indicates the PDOX models of USCS could identify rMETase as an effective therapy of this recalcitrant disease and suggest its clinical development.

Acknowledgement

This study was supported in part by National Cancer Institute grant CA213649.

References

Chello PL, Bertino JR. 1973. Dependence of 5-methyltetrahydrofolate utilization by L5178Y murine leukemia cells in vitro on the presence of hydroxycobalamin and transcobalamin II. *Cancer Res* 33:1898-1904.

Coalson DW, Mecham JO, Stern PH, Hoffman RM. 1982. Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine dependent cancer cells. *Proc Natl Acad Sci USA* 79:4248-4251.

Fu X and Hoffman RM. 1993. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. *Anticancer Res* 1993;13:283-286.

Fu X, Besterman JM, Monosov A, Hoffman, RM. 1991. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci USA* 88:9345-9349.

Fu X, Guadagni F, Hoffman RM. 1992. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. *Proc Natl Acad Sci USA* 89:5645-5649.

Fu X, Le P, Hoffma RM. 1993a. A metastatic-orthotopic transplant nude-mouse model of human patient breast cancer. *Anticancer Res* 13:901-904.

Furukawa T, Kubota T, Watanabe M, Kitajima M, Hoffman RM. 1993. Orthotopic transplantation of histologically intact clinical specimens of stomach cancer to nude mice: correlation of metastatic sites in mouse and individual patient donors. *Int J Cancer* 53:608–612.

Hiroshima Y, Zhao M, Maawy A, Zhang Y, Katz MH, Fleming JB, Uehara F, Miwa S, Yano S, Momiyama M, Suetsugu A, Chishima T, Tanaka K, Bouvet M, Endo I, Hoffman RM. 2014a. Efficacy of *Salmonella typhimurium* A1-R versus chemotherapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX). *J Cell Biochem* 115:1254-1261.

Hiroshima Y, Zhang Y, Murakami T, Maawy A, Miwa S, Yamamoto M, Yano S, Sato S, Momiyama M, Mori R, Matsuyama R, Chishima T, Tanaka K, et al. 2014b. Efficacy of tumor-targeting *Salmonella typhimurium* A1-R in combination with anti-angiogenesis therapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX) and cell line mouse models. *Oncotarget* 5:12346-12357.

Hiroshima Y, Maawy A, Zhang Y, Murakami T, Momiyama M, Mori R, Matsuyama R, Katz MH, Fleming JB, Chishima T, Tanaka K, Ichikawa Y, Endo I, et al. 2014c. Metastatic recurrence in a pancreatic cancer patient derived orthotopic xenograft (PDOX) nude mouse model is inhibited by neoadjuvant chemotherapy in combination with fluorescence-guided surgery with an anti-CA 19–9-conjugated fluorophore. *PLOS ONE* 9:e114310.

Hiroshima Y, Maawy A, Metildi CA, Zhang Y, Uehara F, Miwa S, Yano S, Sato S, Murakami T, Momiyama M, Chishima T, Tanaka K, Bouvet M, et al. 2014d. Successful fluorescence-guided surgery on human colon cancer patient-derived orthotopic xenograft mouse models using a

fluorophore-conjugated anti-CEA antibody and a portable imaging system. J Laparoendosc Adv Surg Tech A 24:241–247.

Hiroshima Y, Maawy AA, Katz MH, Fleming JB, Bouvet M, Endo I, Hoffman RM. 2015a. Selective efficacy of zoledronic acid on metastasis in a patient-derived orthotopic xenograph (PDOX) nude-mouse model of human pancreatic cancer. J Surg Oncol 111:311-315.

Hiroshima Y, Zhang Y, Zhang N, Maawy A, Mii S, Yamamoto M, Uehara F, Miwa S, Yano S, Murakami T, Momiyama M, Chishima T, Tanaka K, et al. 2015b. Establishment of a patient-derived orthotopic xenograph (PDOX) model of HER-2-positive cervical cancer expressing the clinical metastatic pattern. PLOS ONE 10:e0117417.

Hiroshima Y, Zhang Y, Zhang N, Uehara F, Maawy A, Murakami T, Mii S, Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, Matsuyama R, et al. 2015c. Patient-derived orthotopic xenograft (PDOX) nude mouse model of soft-tissue sarcoma more closely mimics the patient behavior in contrast to the subcutaneous ectopic model. Anticancer Res 35:697–701.

Hiroshima Y, Zhao M, Zhang Y, Zhang N, Maawy A, Murakami T, Mii S, Uehara F, Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, et al. 2015d. Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice. PLoS One 10:e0134324.

Hoffman RM. 2015. Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: A 40-year odyssey. Expert Opin

Biol Ther 15:21-31.

Hoffman RM. 2017. The wayward methyl group and the cascade to cancer. Cell Cycle 16, 825-829.

Hoffman RM and Erbe RW. 1976. High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci USA 73:1523-1527.

Hoffman RM and Jacobsen SJ. 1980. Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc Natl Acad Sci USA 77:7306-7310.

Hoffman RM, Jacobsen SJ, Erbe RW. 1978. Reversion to methionine independence by malignant rat and SV40-transformed human fibroblasts. Biochem Biophys Res Commun 82:228-234.

Hoffman RM, Jacobsen SJ, Erbe RW. 1979. Reversion to methionine independence in simian virus 40-transformed human and malignant rat fibroblasts is associated with altered ploidy and altered properties of transformation. Proc Natl Acad Sci USA 76:1313-1317.

Katz MH, Takimoto S, Spivack D, Moossa AR, Hoffman RM, Bouvet M. 2003. A novel red fluorescent protein orthotopic pancreatic cancer model for the preclinical evaluation of chemotherapeutics. J Surg Res 113:151-160.

Igarashi K, Kawaguchi K, Murakami T, Kiyuna T, Miyake K, Singh A, Nelson SD, Dry SM, Li Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Tsuchiya H, Eilber FC, Hoffman RM. 2017a. 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. J Cell Biochem 118:2739-2743.

Igarashi K, Kawaguchi K, Kiyuna T, Murakami T, Miwa S, Nelson S,D, Dry S,M, Li Y, Singh A, Kimura H, Hayashi K, Yamamoto N, Tsuchiya H, et al. 2017b. Temozolomide combined with irinotecan caused regression in an adult pleomorphic rhabdomyosarcoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. Oncotarget, Epub ahead of print. DOI: 10.18632/oncotarget.16548.

Igarashi K, Kawaguchi K, Kiyuna T, Murakami T, Miwa S, Nelson SD, Dry SM, Li Y, Singh A, Kimura H, Hayashi K, Yamamoto N, Tsuchiya H, et al. 2017c. Patient-derived orthotopic xenograft (PDOX) mouse model of adult rhabdomyosarcoma invades and recurs after resection in contrast to the subcutaneous ectopic model. Cell Cycle 16:91-94.

Igarashi K, Kawaguchi K, Murakami T, Kiyuna T, Miyake K, Nelson SD, Dry SM, Li Y, Yanagawa J, Russell TA, Singh A, Yamamoto N, Hayashi K, et al. 2017d. Intra-arterial administration of tumor-targeting *Salmonella typhimurium* A1-R regresses a cisplatin-resistant relapsed osteosarcoma in a patient-derived orthotopic xenograft (PDOX) mouse model. Cell Cycle 16:1164-1170.

Judde JG, Ellis M, Frost P. 1989. Biochemical analysis of the role of transmethylation in the

methionine dependence of tumor cells. Cancer Res 49:4859-4865.

Kawaguchi K, Murakami T, Chmielowski B, Igarashi K, Kiyuna T, Unno M, Nelson SD, Russell TA, Dry SM, Li Y, Eilber FC, Hoffman RM. 2016a. Vemurafenib-resistant BRAF-V600E mutated melanoma is regressed by MEK targeting drug trametinib, but not cobimetinib in a patient-derived orthotopic xenograft (PDOX) mouse model. Oncotarget 7:71737-71743.

Kawaguchi K, Igarashi K, Murakami T, Chmiewloski B, Kiyuna T, Zhao M, Zhang Y, Singh A, Unno M, Nelson S,D, Russell T, Dry SM, Li Y, et al. 2016b. Tumor-targeting *Salmonella typhimurium* A1-R combined with Temozolomide regresses malignant melanoma with a BRAF-V600 mutation in a patient-derived orthotopic xenograft (PDOX) model. Oncotarget 7:85929-85936.

Kawaguchi K, Igarashi K, Murakami T, Kiyuna T, Lwin T, Hwang H-K, DeLong J, Clary B, Bouvet M, Unno M, Hoffman RM. 2017a. MEK inhibitors cobimetinib and trametinib, regressed a gemcitabine-resistant pancreatic cancer patient-derived orthotopic xenograft (PDOX). Oncotarget, Epub ahead of print. DOI: 10.18632/Oncotarget.17667.

Kawaguchi K, Igarashi K, Murakami T, Kiyuna T, Nelson SD, Dry SM, Li Y, Russell TA, Singh AS, Chmielowski B, Unno M, Eilber FC, Hoffman RM. 2017b. Combination of gemcitabine and docetaxel regresses both gastric leiomyosarcoma proliferation and invasion in an imageable patient-derived orthotopic xenograft (iPDOX) model. Cell Cycle 16:1063-1069.

Kawaguchi K, Igarashi K, Murakami T, Kiyuna T, Zhao M, Zhang Y, Nelson SD, Russell TA, Dry SM, Singh AS, Chmielowski B, Li Y, Unno M, et al. 2017c. *Salmonella typhimurium* A1-R targeting of a chemotherapy resistant BRAF-V600E melanoma in a patient-derived orthotopic xenograft (PDOX) model is enhanced in combination with either vemurafenib or temozlomide. Cell Cycle, Epub ahead of print. DOI: 10.1080/15384101.2017.1314420.

Kawaguchi K, Igarashi K, Murakami T, Zhao M, Zhang Y, Chmielowski B, Kiyuna T, Nelson SD, Russell TA, Dry SM, Li Y, Unno M, Eilber FC, et al. 2017d. Tumor-targeting *Salmonella typhimurium* A1-R sensitizes melanoma with a BRAF-V600E mutation to vemurafenib in a patient-derived orthotopic xenograft (PDOX) nude mouse model. J Cell Biochem., Epub ahead of print. DOI: 10.1002/jcb.25886.

Kiyuna T, Murakami T, Tome Y, Kawaguchi K, Igarashi K, Zhang Y, Zhao M, Li Y, Bouvet M, Kanaya F, Singh A, Dry S, Eilber FC, et al. 2016. High efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a doxorubicin- and dactolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model. Oncotarget 7:33046-33054.

Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM. 1983. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem Biophys Res Commun 117:429-434.

Metildi CA, Kaushal S, Luiken GA, Talamini MA, Hoffman RM, Bouvet M. 2014. Fluorescently-labeled chimeric anti-CEA antibody improves detection and resection of human

colon cancer in a patient-derived orthotopic xenograft (PDOX) nude mouse model. J Surg Oncol 109:451–458.

Murakami T, Singh AS, Kiyuna T, Dry SM, Li Y, James AW, Igarashi K, Kawaguchi K, DeLong JC, Zhang Y, Hiroshima Y, Russell T, Eckardt MA, et al. 2016a. Effective molecular targeting of CDK4/6 and IGF-1R in a rare FUS-ERG fusion CDKN2A-deletion doxorubicin-resistant Ewing's sarcoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. Oncotarget 7:47556-47564.

Murakami T, DeLong J, Eilber FC, Zhao M, Zhang Y, Zhang N, Singh A, Russell T, Deng S, Reynoso J, Quan C, Hiroshima Y, Matsuyama R, et al. 2016b. Tumor-targeting *Salmonella typhimurium* A1-R in combination with doxorubicin eradicate soft tissue sarcoma in a patient-derived orthotopic xenograft PDOX model. Oncotarget 7:12783-12790.

Murakami T, Li S, Han Q, Tan Y, Kiyuna T, Igarashi K, Kawaguchi K, Hwang HK, Miyaki K, Singh AS, Nelson SD, Dry SM, Li Y, Hiroshima Y, Lwin TM, DeLong JC, Chishima T, Tanaka K., Bouvet M, Endo I, Eilber FC, Hoffman RM. 2017a. Recombinant methioninase effectively targets a Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model. Oncotarget 8:35630-35638.

Murakami T, Kiyuna T, Kawaguchi K, Igarashi K, Singh AS, Hiroshima Y, Zhang Y, Zhao M, Miyake K, Nelson SD, Dry SM, Li Y, DeLong JC, et al. 2017b. The irony of highly-effective bacterial therapy of a patient-derived orthotopic xenograft (PDOX) model of Ewing's sarcoma, which was blocked by Ewing himself 80 years ago. Cell Cycle 16:1046-1052.

Murakami T, Igarashi K, Kawaguchi K, Kiyuna T, Zhang Y, Zhao M, Hiroshima Y, Nelson SD, Dry SM, Li Y, Yanagawa J, Russell T, Federman N, et al. 2017c. Tumor-targeting *Salmonella typhimurium* A1-R regresses an osteosarcoma in a patient-derived xenograft model resistant to a molecular-targeting drug. *Oncotarget* 8:8035-8042.

Reid R, de Silva MV, Paerson L, Ryan E, Fisher C. 2003. Low grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes share a common t(7; 16) (q 34;p11) translocation. *Am J Surg Pathol* 27:1229-1236.

Shaikh S, Katke RD, Raut SH. 2014. Spindle cell sarcoma arising from nerve sheath presenting as huge abdominal mass. *J Case Reports* 4:232-235.

Stern PH, Mecham JO, Wallace CD, Hoffman RM. 1983. Reduced free-methionine in methionine-dependent SV40-transformed human fibroblasts synthesizing apparently normal amounts of methionine. *J Cell Physiol* 117:9-14.

Stern PH, Hoffman RM. 1984. Elevated overall rates of transmethylation in cell lines from diverse human tumors. *In Vitro – Rapid Commun. in Cell Biology* 20:663-670.

Sugimura T, Birnbaum SM, Winitz M, Greenstein JP. 1959. Quantitative nutritional studies with water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one essential amino acid. *Arch Biochem Biophys* 81:448-455.

Sun X, Tan Y, Yang Z, Li S, Hoffman RM. 2005. A rapid HPLC method for the measurement of ultra-low plasma methionine concentrations applicable to methionine depletion therapy. *Anticancer Res* 24:59-62.

Tan Y, Xu M, Tan X-Z, Tan X-Y, Wang, X, Saikawa Y, Nagahama T, Sun X, Lenz M, Hoffman RM. 1997. Overexpression and large-scale production of recombinant L-methionine- α -deamino- γ -mercaptomethane-lyase for novel anticancer therapy. *Prot Exp Purification* 9:233-245.

Tan Y, Xu M, Hoffman RM. 2010. Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells in vitro. *Anticancer Res* 30:1041-1046.

Wang X, Fu X, Hoffman RM. 1992. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. *Int J Cancer* 51:992-995.

Yamamoto M, Zhao M, Hiroshima Y, Zhang Y, Shurell E, Eilber F,C, Bouvet M, Noda M, Hoffman RM. 2016. Efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. *PLoS One* 11:e0160882.

Yano S, Li S, Han Q, Tan Y, Bouvet M, Fujiwara T, Hoffman RM. 2014. Selective methioninase-induced trap of cancer cells in S/G2 phase visualized by FUCCI imaging confers chemosensitivity. *Oncotarget* 5:8729-8736.

Figure Legends

Figure 1. Treatment schema.

Figure 2. Efficacy of doxorubicin (DOX) and L-methionine α -deamino- γ -mercaptomethane lyase (recombinant methioninase [rMETase]) on an undifferentiated spindle-cell sarcoma (USCS) PDOX. A USCS from a patient was grown orthotopically in the right biceps femoris muscle of nude mice and allowed to form tumors. Mice were treated with DOX (3 mg/kg/week, i.p., for 2 weeks) or rMETase (100 U/mouse/day, i.p., for 14 days). Representative photographs of mice from each group at day-14.

Figure 3. Growth curves of the untreated and DOX and rMETase-treated USCS PDOX. Tumor volume was measured at the indicated time points after the onset of treatment. N=8 mice/group. * $p < 0.001$

Figure 4. Body weight of untreated control, DOX and rMETase-treated groups. Bar graph shows body weight in each group at pre-treatment and 2 weeks after drug administration. There were no significant differences between each group.

Figure 5. L-methionine levels in pre- and post-rMETase treatment of the USCS PDOX. Bar graphs show tumor L-methionine level normalized by tumor protein concentration. Tumor L-methionine levels in the post-rMETase-treated animals were lower than control. Error bars: $\pm$ SD. N = 4 for each group.

Figure 6. Tumor histology. Hematoxylin and eosin (H&E)-stained section of the original

patient's tumor (**A**), untreated PDOX tumor (**B**), PDOX tumor treated with DOX (**C**), PDOX tumor treated with rMETase (**D**). Scale bars: 80 μ m.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6