

UCLA

UCLA Previously Published Works

Title

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.

Permalink

<https://escholarship.org/uc/item/9jg9q592>

Journal

Cell cycle (Georgetown, Tex.), 16(12)

ISSN

1538-4101

Authors

Igarashi, Kentaro
Kawaguchi, Kei
Murakami, Takashi
et al.

Publication Date

2017-06-01

DOI


10.1080/15384101.2017.1317417

Peer reviewed

REPORT



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

Kentaro Igarashi ^{a,b,c}, Kei Kawaguchi^{a,b}, Takashi Murakami^{a,b}, Tasuku Kiyuna^{a,b}, Kentaro Miyake^{a,b}, Scott D. Nelson^d, Sarah M. Dry^d, Yunfeng Li^d, Jane Yanagawa^e, Tara A. Russell ^e, Arun S. Singh^f, Norio Yamamoto^c, Katsushi Hayashi^c, Hiroaki Kimura^c, Shinji Miwa^c, Hiroyuki Tsuchiya^c, Fritz C. Eilber ^e, and Robert M. Hoffman^{a,b}

^aAntiCancer, Inc., San Diego, CA, USA; ^bDepartment of Surgery, University of California, San Diego, CA, USA; ^cDepartment of Orthopaedic Surgery, Kanazawa University, Kanazawa, Japan; ^dDepartment of Pathology, University of California Los Angeles, CA, USA; ^eDivision of Surgical Oncology, University of California, Los Angeles, CA, USA; ^fDivision of Hematology-Oncology, University of California, Los Angeles, CA, USA

ABSTRACT

Previously, a patient-derived orthotopic xenograft (PDOX) model was established with a lung metastasis from an osteosarcoma patient which developed after adjuvant cisplatin (CDDP) treatment. In this model, we previously demonstrated the efficacy of trabectedin (TRAB) and temozolomide (TEM) compared with CDDP. In the present report, osteosarcoma tissue was implanted orthotopically in the distal femur of mice which were randomized into the following groups when tumor volume reached approximately 100 mm³; On day 14 after initiation of treatment, all but CDDP significantly inhibited tumor volume growth compared with untreated controls. Control (G1): 793.7 ± 215.0 mm³; CDDP (G2): 588.1 ± 176.9 mm³; *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) intravenous (i.v.) (G3): 269.7 ± 72.7 mm³; *S. typhimurium* A1-R intra-arterial (i.a.) (G4): 70.2 ± 18.9 mm³ (CDDP: p = 0.056; *S. typhimurium* A1-R i.v.: p = 0.0001; *S. typhimurium* A1-R i.a.: p = 0.00003, all vs. untreated controls). i.a. administration of *S. typhimurium* A1-R was significantly more effective than either CDDP (p = 0.00007), or i.v. administration of *S. typhimurium* A1-R (p = 0.00007) and significantly regressed the tumor volume compared with day 0 (p = 0.001). The new model of i.a. administration of *S. typhimurium* A1-R has great promise for the treatment of recalcitrant osteosarcoma.

ARTICLE HISTORY

Received 10 March 2017
Accepted 3 April 2017

KEYWORDS

Osteosarcoma; PDOX; *Salmonella typhimurium* A1-R; intra-arterial; cisplatin-resistant; recurrent

Introduction

Toward the goal of precision oncology, our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI), including pancreatic,^{1–4} breast,⁵ ovarian,⁶ lung,⁷ cervical,⁸ colon,^{9–11} and stomach cancer,¹² sarcoma,^{13–17} and melanoma.^{18–20}

In a previous study, we evaluated the efficacy of trabectedin (TRAB) and temozolomide (TEM) compared with cisplatin (CDDP) on a lung-metastatic osteosarcoma PDOX mouse model.²¹ TEM and TRAB, but not CDDP, significantly inhibited tumor volume compared with the untreated controls. The results of the previous study showed that a PDOX model of an osteosarcoma lung-metastasis that recurred after adjuvant CDDP-treatment, had identified potentially, highly-effective drugs for this recalcitrant disease, while precisely maintaining the CDDP resistance of the tumor in the patient.²¹

However, more effective therapeutics are needed for metastatic osteosarcoma. Toward this goal, our laboratory developed tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R). *S. typhimurium* A1-R was effective against primary

and metastatic tumors as monotherapy in nude mouse models of major cancers, including prostate,^{22,23} breast,^{24–26} lung,^{27,28} pancreatic,^{1,29–32} ovarian,^{33, 34} stomach,³⁵ and cervical cancer.³⁶ In addition, *S. typhimurium* A1-R was effective against patient-derived orthotopic models (PDOX) of pancreatic cancer,^{1,32} sarcoma,^{13,15,37} and melanoma.^{18–20}

Metastatic osteosarcoma is a recalcitrant disease. We previously reported that a patient-derived subcutaneous-transplant nude-mouse model of the osteosarcoma lung metastasis that occurred after adjuvant CDDP treatment, was regressed by tumor-targeting *S. typhimurium* A1-R. The osteosarcoma was only partially sensitive to the molecular-targeting drug sorafenib, which did not arrest its growth. *S. typhimurium* A1-R was significantly more effective than sorafenib.^{21, 37}

We previously reported the efficacy and safety of intra-portal-vein (iPV) targeting of *S. typhimurium* A1-R on colon cancer liver metastasis in a nude-mouse orthotopic model.³⁸

In the present report, we demonstrate the high efficacy of intra-arterial (i.a.) administration of *S. typhimurium* A1-R on the CDDP-resistant, recurrent osteosarcoma in the PDOX model.

Results and discussion

Distribution of *S. typhimurium* A1-R in the CDDP-resistant relapsed osteosarcoma PDOX mouse model

Distribution of *S. typhimurium* A1-R was imaged by confocal microscopy with the Olympus FV1000 on day 3 after injection of *S. typhimurium* A1-R (Fig. 1). The mean fluorescence intensity in the PDOX tumor on day 3 after intra-arterial (i.a.) injection of *S. typhimurium* A1-R was 1.76×10^6 compared with 1.49×10^5 after intravenous (i.v.) injection ($p = 0.000013$; Fig. 2A). The fluorescence area in the PDOX tumor on day 3 after i.a. injection of *S. typhimurium* A1-R was $485.4 \pm 103.7 \mu\text{m}^2$ compared with $51.3 \pm 45.7 \mu\text{m}^2$ after i.v. injection ($p < 0.00001$; Fig. 2B). In the tibialis anterior muscle of the tumor-bearing limb, fluorescence intensity after i.a. injection *S. typhimurium* A1-R was 5.18 compared with 2.74 after i.v. injection ($p = 0.50$). The fluorescence area after i.a. injection *S. typhimurium* A1-R was $0.01 \mu\text{m}^2$ compared with $0.02 \mu\text{m}^2$ after i.v. injection in the tibialis anterior muscle of affected limb ($p = 0.42$).

Efficacy of CDDP, *S. typhimurium* A1-R i.v. and *S. typhimurium* A1-R i.a. on the CDDP-resistant osteosarcoma PDOX mouse model

On day 14 after initiation of treatment, all but CDDP significantly inhibited tumor volume growth compared with

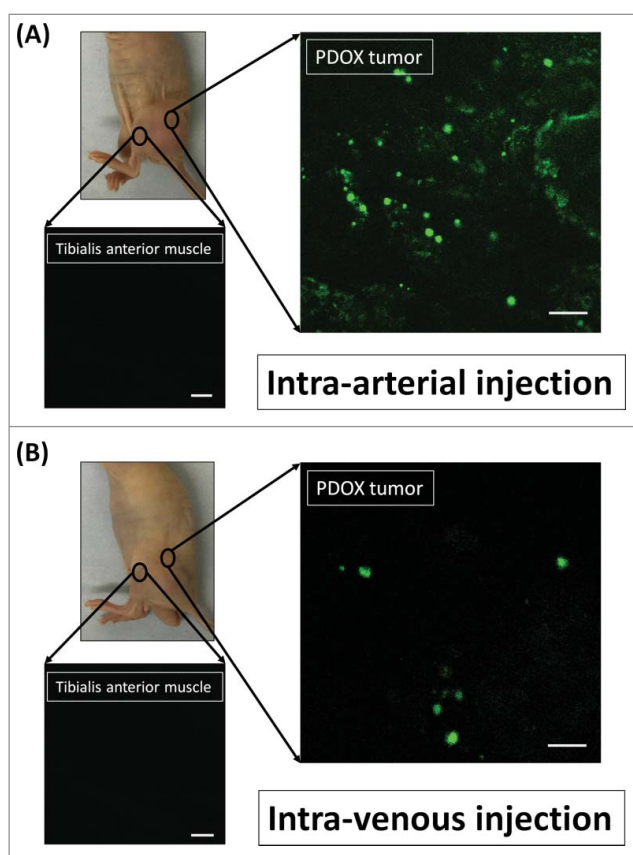


Figure 1. Distribution of fluorescence imaging. (A) *S. typhimurium*-A1-R-GFP targeting the osteosarcoma PDOX after intra-arterial (i.a.) injection. (B) *S. typhimurium* A1-R-GFP targeting the osteosarcoma after intravenous (i.v.) injection. Confocal microscopy imaging with the Olympus FV1000 demonstrated *S. typhimurium* A1-R-GFP targeting the osteosarcoma PDOX. Scale bars: $12.5 \mu\text{m}$.

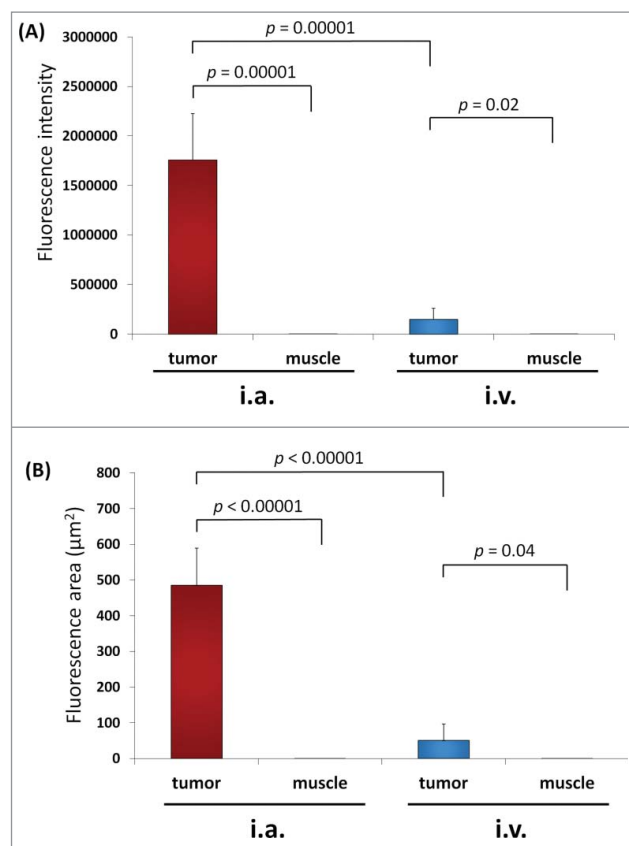


Figure 2. (A) Fluorescence intensity of *S. typhimurium* A1-R-GFP targeting the osteosarcoma PDOX after i.a. or i.v. administration. (B) Fluorescent area of *S. typhimurium* A1-R-GFP targeting the osteosarcoma PDOX after i.a. or i.v. administration.

untreated control (G1): $793.7 \pm 215.0 \text{ mm}^3$; CDDP (G2): $588.1 \pm 176.9 \text{ mm}^3$; *S. typhimurium* A1-R i.v. (G3): $269.7 \pm 72.7 \text{ mm}^3$; *S. typhimurium* A1-R i.a. (G4): $70.2 \pm 18.9 \text{ mm}^3$ (CDDP: $p = 0.056$; *S. typhimurium* A1-R i.v.: $p = 0.0001$; *S. typhimurium* A1-R i.a.: $p = 0.00003$, all vs. untreated controls). i.a. administration of *S. typhimurium* A1-R was significantly more effective than either CDDP ($p = 0.00007$); i.v. administration of *S. typhimurium* A1-R ($p = 0.00007$) and significantly regressed the tumor volume compared with day 0 ($p = 0.001$) (Fig. 3).

There were no animal deaths, limb necrosis or paraplegia in any group. The body weight of treated mice were not significantly different in each group (Fig. 4).

Histology of original tumor and PDOX tumors

High power photomicrography of the original patient tumor demonstrated a chondroid matrix occupied by anaplastic cells. The tumor had hypercellular areas populated by anaplastic cells displaying nuclear pleomorphism, coarse and hyperchromatic chromatin and abundant mitotic figures (Fig. 5A). High power photomicrography of the untreated PDOX tumor showed the tumor had a solid and chondroblastic appearance similar to the original patient tumor and had hypercellular areas filled with cancer cells displaying nuclear pleomorphism and mitotic figures (Fig. 5B). The PDOX tumor treated with CDDP comprised viable cells without apparent necrosis or inflammatory changes and had almost the same features as the untreated control (Fig. 5C).

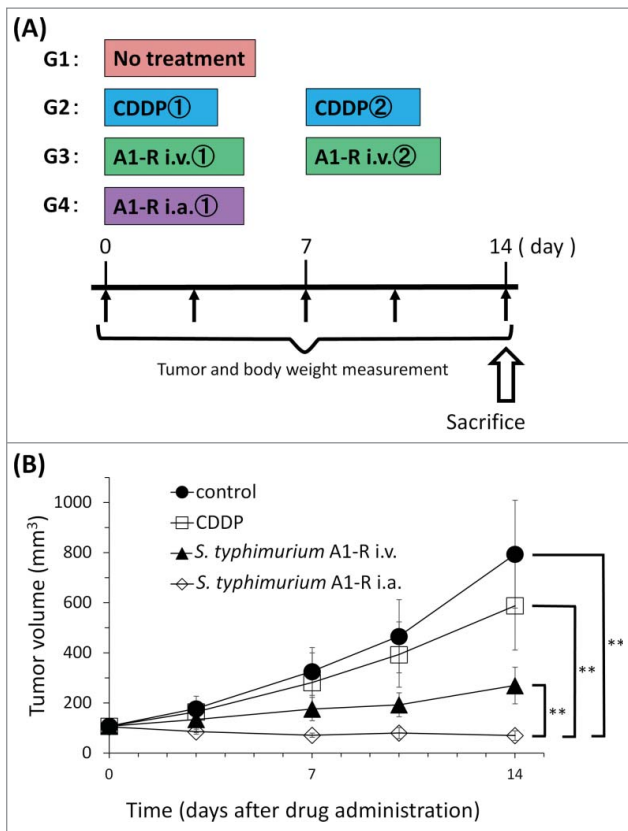


Figure 3. (A) Treatment schema. Mice were treated with CDDP, *S. typhimurium* A1-R i.v. or *S. typhimurium* A1-R i.a. CDDP (6 mg/kg/week i.p. for 2 weeks); *S. typhimurium* A1-R i.v. (5×10^7 CFU/100 μ l, i.v., weekly, for 2 weeks); *S. typhimurium* A1-R i.a. (5×10^5 CFU/100 μ l, i.a., once). Tumor volume was measured at the indicated time points after the onset of treatment. $n = 8$ mice/group. (B) Treatment efficacy. * $p < 0.05$, ** $p < 0.001$.

The PDOX Tumor treated with i.v. injection of *S. typhimurium* A1-R showed changes in cancer-cell shape with a necrotic area (Fig. 5D). The i.a. injection of *S. typhimurium* A1-R-treated tumor showed more extensive tumor necrosis (Fig. 5E).

Previously-developed concepts and strategies of highly-selective tumor targeting can take advantage of molecular

targeting of tumors, including tissue-selective therapy which focuses on unique differences between normal and tumor tissues.³⁹⁻⁴⁴

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-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 Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873–1. To minimize any suffering of the animals, 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 killed by CO₂ inhalation when they met the following humane end point criteria: severe tumor burden (more than 20 mm in diameter), prostration, significant body weight loss, difficulty breathing, rotational motion and body temperature drop.

Patient-derived tumor

The study was previously reviewed and approved by the UCLA Institutional Review Board (IRB #10–001857).³⁷ Written informed consent was obtained from the patient as part of the above-mentioned UCLA Institutional Review Board-approved protocol. A 16-year old patient with localized left distal femoral high grade osteosarcoma underwent CDDP-based neo-adjuvant chemotherapy and limb salvage with distal femoral replacement.

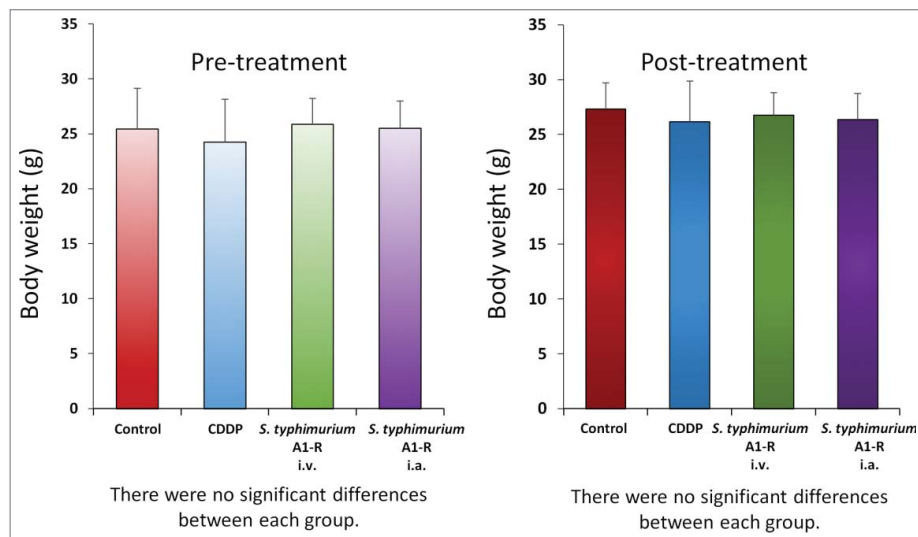


Figure 4. Body weight after various treatments. Bar graphs show body weight in each group at pre-treatment and 2 weeks after drug administration.

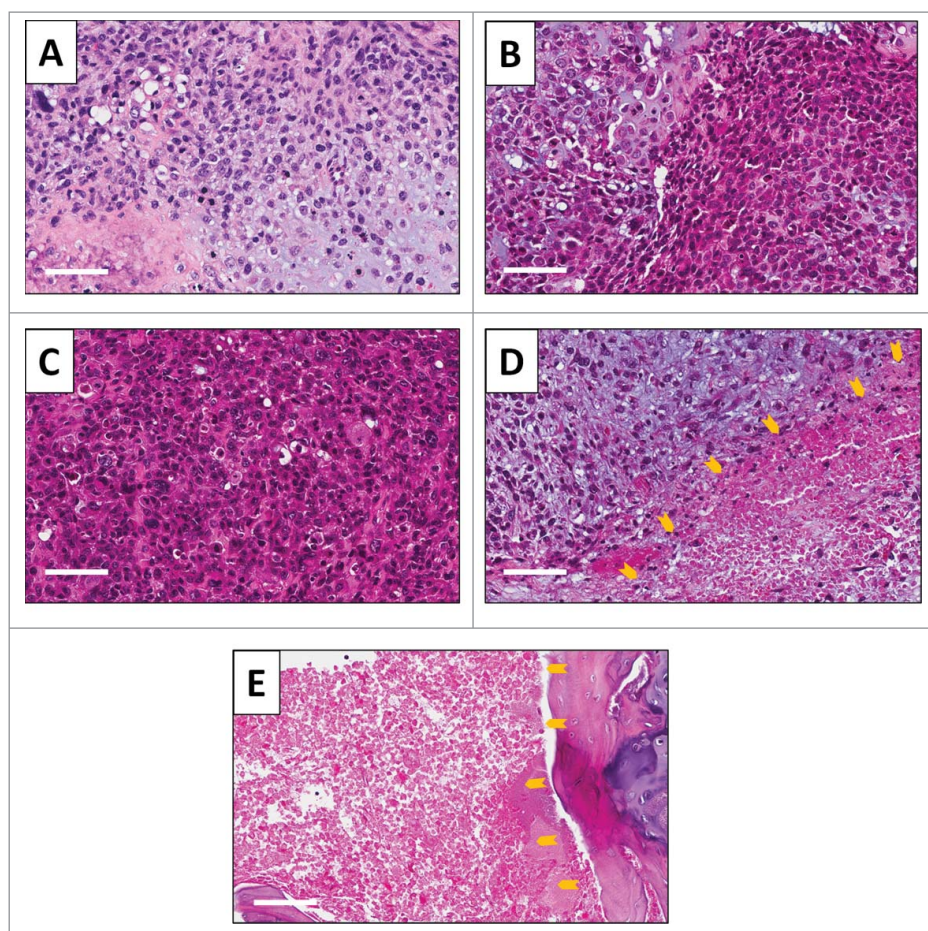


Figure 5. Tumor histology. Hematoxylin and eosin (H&E)-stained section of the (A) original patient tumor; (B) untreated PDOX tumor; (C) PDOX tumor treated with CDDP; (D) PDOX tumor treated with *S. typhimurium* A1-R i.v.; and (E) PDOX tumor treated with *S. typhimurium* A1-R i.a.. Necrotic areas are indicated by yellow arrows. White scale bars: 80 μm .

One year later, the osteosarcoma recurred with 3 bilateral metachronous pulmonary metastases. The patient was treated with curative surgery at the Division of Surgical Oncology, University of California, Los Angeles (UCLA). The patient did not receive chemotherapy or radiotherapy before lung surgery.³⁷

SOI for establishment of the PDOX osteosarcoma model

The osteosarcoma from the patient was previously established subcutaneously in mice.³⁷ Subcutaneously grown tumors were harvested and cut into small fragments (3–4 mm). After nude mice were anesthetized, a 10 mm skin incision was made on the right thigh, the vastus lateralis muscle was opened and the biceps femoris muscle was split to reach the distal femur. An incision was made in the lateral patello-femoral ligament, sparing the knee joint and then the lateral condyle of the femur was resected. A single 3 to 4 mm tumor fragment was implanted orthotopically into this space to establish a PDOX model. The muscle and wound was closed with 6–0 nylon suture (Ethilon, Ethicon, Inc., NJ, USA) (Fig. 1).

Treatment design

Primary osteosarcomas are fed by an artery. Therefore, in the present study, we compared i.a. administration of *S. typhimurium* A1-R with i.v. administration in the osteosarcoma PDOX.

The PDOX models were randomized into the following groups when tumor volume reached 100 mm³: G1, control without treatment, n = 8; G2, CDDP (6 mg/kg, intraperitoneal (i.p.) injection, weekly, for 2 weeks, n = 8); G3, i.v. injection of *S. typhimurium* A1-R (5×10^7 CFU/100 μl , i.v., weekly, for 2 weeks, n = 8); G4, i.a. injection of *S. typhimurium* A1-R (5×10^5 CFU/100 μl , i.a., once, n = 8). Tumor length, width and mouse body weight were measured twice in a week. Tumor volume was calculated by following formula: Tumor volume (mm³) = length (mm) \times width (mm) \times width (mm) \times 1/2. Data are presented as mean \pm SD.

Preparation and administration of *S. typhimurium* A1-R

GFP-expressing *S. typhimurium* A1-R bacteria (AntiCancer Inc.,) were grown overnight in LB medium (Fisher Sci., Hanover Park, IL, USA) and then diluted 1:10 in LB medium. Bacteria were harvested at late-log phase, washed with PBS, and then diluted in PBS.^{22–24}

i.a. injection of *S. typhimurium* A1-R

Nude mice were anesthetized with the ketamine mixture as described above and placed in a right lateral decubitus position. A 20 mm skin incision was made on the left lateral abdomen followed by exposure of the abdominal aorta. *S. typhimurium*

A1-R (5×10^5 CFU in 100 μ l PBS) was injected in the abdominal aorta using a 31G needle. After removal of the needle, bleeding was stopped by gently pressing the puncture site with a cotton swab. After injection, the abdomen was closed with non-absorbable sutures.

Confocal microscopy

The FV1000 confocal microscope (Olympus, Tokyo, Japan) was used for high-resolution imaging. Fluorescence images were obtained using the $20 \times /0.50$ UPlan FLN and $40 \times /1.3$ oil Olympus UPLAN FLN objectives.⁴⁵

Distribution of *S. typhimurium* A1-R

Twelve PDOX mouse models were treated with i.v. administration of *S. typhimurium* A1-R (5×10^7 CFU/100 μ l, i.v., once) or i.a. administration of *S. typhimurium* A1-R (5×10^5 CFU/100 μ l, i.a., once) when the tumor volume reached 500 mm³, (n = 6 mice each). PDOX tumors or the tibialis anterior muscle of the affected limb were resected on day 3. The distribution of *S. typhimurium* A1-R was determined by confocal imaging with the FV1000.⁴⁶ Three random fields were accessed in each specimen.

Histological analysis

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (3 μ m) 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).

Conclusions

The new model of i.a. administration of *S. typhimurium* A1-R has great promise for the treatment of recalcitrant osteosarcoma.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Kentaro Igarashi  <http://orcid.org/0000-0002-4680-4317>

Tara A. Russell  <http://orcid.org/0000-0002-3912-3601>

Fritz C. Eilber  <http://orcid.org/0000-0003-3336-9333>

References

- [1] Hiroshima Y, Zhang Y, Murakami T, Maawy AA, Miwa S, Yamamoto M, Yano S, Sato S, Momiyama M, Mori R, et al. 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* 2014; 5:12346-57; PMID:25402324; <https://doi.org/10.18632/oncotarget.2641>
- [2] Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. *Proc Natl Acad Sci USA* 1992; 89:5645-9; PMID:1608975; <https://doi.org/10.1073/pnas.89.12.5645>
- [3] Hiroshima Y, Maawy A, Zhang Y, Murakami T, Momiyama M, Mori R, Matsuyama R, Katz MH, Fleming JB, Chishima T, et al. 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* 2014; 9:e114310; PMID:25463150; <https://doi.org/10.1371/journal.pone.0114310>
- [4] Hiroshima Y, Maawy AA, Katz MH, Fleming JB, Bouvet M, Endo I, Hoffman RM. Selective efficacy of zoledronic acid on metastasis in a patient-derived orthotopic xenograft (PDOX) nude-mouse model of human pancreatic cancer. *J Surg Oncol* 2015; 111:311-15; PMID:25394368; <https://doi.org/10.1002/jso.23816>
- [5] Fu X, Le P, Hoffman RM. A metastatic-orthotopic transplant nude-mouse model of human patient breast cancer. *Anticancer Res* 1993; 13:901-4; PMID:8352558
- [6] Fu X, Hoffman RM. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. *Anticancer Res* 1993; 13:283-6; PMID:8517640
- [7] Wang X, Fu X, Hoffman RM. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. *Int J Cancer* 1992; 51:992-5; PMID:1639545; <https://doi.org/10.1002/ijc.2910510621>
- [8] Hiroshima Y, Zhang Y, Zhang M, Maawy A, Mii S, Yamamoto M, Uehara F, Miwa S, Yano S, Murakami T, et al. Establishment of a patient-derived orthotopic xenograft (PDOX) model of HER-2-positive cervical cancer expressing the clinical metastatic pattern. *PLOS ONE* 2015; 10:e0117417; PMID:25689852; <https://doi.org/10.1371/journal.pone.0117417>
- [9] Fu X, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci USA* 1991; 88:9345-9; PMID:1924398; <https://doi.org/10.1073/pnas.88.20.9345>
- [10] Metildi CA, Kaushal S, Luiken GA, Talamini MA, Hoffman RM, Bouvet M. 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* 2014; 109:451-8; PMID:24249594; <https://doi.org/10.1002/jso.23507>
- [11] Hiroshima Y, Maawy A, Metildi CA, Zhang Y, Uehara F, Miwa S, Yano S, Sato S, Murakami T, Momiyama M, et al. 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* 2014; 24:241-7; PMID:24494971; <https://doi.org/10.1089/lap.2013.0418>
- [12] Furukawa T, Kubota T, Watanabe M, Kitajima M, Hoffman RM. 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* 1993; 53:608-12; PMID:8436434; <https://doi.org/10.1002/ijc.2910530414>
- [13] Murakami T, DeLong J, Eilber FC, Zhao M, Zhang Y, Zhang N, Singh A, Russell T, Deng S, Reynoso J, et al. Tumor-targeting *Salmonella typhimurium* A1-R in combination with doxorubicin eradicate soft tissue sarcoma in a patient-derived orthotopic xenograft PDOX model. *Oncotarget* 2016; 7:12783-90; PMID:26859573
- [14] Hiroshima Y, Zhao M, Zhang Y, Zhang N, Maawy A, Murakami T, Mii S, Uehara F, Yamamoto M, Miwa S, Yano S, et al. Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemoresistant patient soft-tissue sarcoma in nude mice. *PLoS One* 2015; 10:e0134324; PMID:26237416; <https://doi.org/10.1371/journal.pone.0134324>
- [15] Kiyuna T, Murakami T, Tome Y, Kawaguchi K, Igarashi K, Zhang Y, Zhao M, Li Y, Bouvet M, Kanaya F, et al. High efficacy of tumor-

- targeting *Salmonella typhimurium* A1-R on a doxorubicin- and dac-tolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model. *Oncotarget* 2016; 7:33046-54; PMID:27105519
- [16] Murakami T, Singh AS, Kiyuna T, Dry SM, Li Y, James AW, Igarashi K, Kawaguchi K, DeLong JC, Zhang Y, et al. Effective molecular targeting of CDK4/6 and IGF-1R in a rare FUS-ERG fusion CDKN2A-deletion doxorubicin-resistant Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model. *Oncotarget* 2016; 7:47556-64; PMID:27286459
- [17] Hiroshima Y, Zhang Y, Zhang N, Uehara F, Maawy A, Murakami T, Mii S, Yamamoto M, Miwa S, Yano S, et al. 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* 2015; 35:697-701; PMID:25667448
- [18] Yamamoto M, Zhao M, Hiroshima Y, Zhang Y, Shurell E, Eilber FC, Bouvet M, Noda M, Hoffman RM. Efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. *PLoS One* 2016; 11:e0160882; PMID:27500926; <https://doi.org/10.1371/journal.pone.0160882>
- [19] Kawaguchi K, Murakami T, Chmielowski B, Igarashi K, Kiyuna T, Unno M, Nelson SD, Russell TA, Dry SM, Li Y, et al. 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* 2016; 7:71737-43; PMID:27690220
- [20] Kawaguchi K, Igarashi K, Murakami T, Chmielowski B, Kiyuna T, Zhao M, Zhang Y, Singh A, Unno M, Nelson SD, et al. 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* 2016; 7:85929-36; PMID:27835903
- [21] Igarashi K, Murakami T, Kawaguchi K, Kiyuna T, Miyake K, Zhang Y, Nelson SD, Dry SM, Li Y, Yanagawa J, et al. A patient-derived orthotopic xenograft (PDOX) mouse model of an osteosarcoma lung metastasis that recurred after cisplatin treatment was resistant to cisplatin but highly-sensitive to temozolomide and trabectedin. *JCO Precision Oncol*, submitted
- [22] Zhao M, Yang M, Li X-M, Jiang P, Baranov E, Li S, Xu M, Penman S, Hoffman RM. Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing *Salmonella typhimurium*. *Proc Natl Acad Sci USA* 2005; 102:755-60; PMID:15644448; <https://doi.org/10.1073/pnas.0408422102>
- [23] Zhao M, Geller J, Ma H, Yang M, Penman S, Hoffman RM. Monotherapy with a tumor-targeting mutant of *Salmonella typhimurium* cures orthotopic metastatic mouse models of human prostate cancer. *Proc Natl Acad Sci USA* 2007; 104:10170-4; PMID:17548809; <https://doi.org/10.1073/pnas.0703867104>
- [24] Zhao M, Yang M, Ma H, Li X, Tan X, Li S, Yang Z, Hoffman RM. Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. *Cancer Res* 2006; 66:7647-52; PMID:16885365; <https://doi.org/10.1158/0008-5472.CAN-06-0716>
- [25] Zhang Y, Tome Y, Suetsugu A, Zhang L, Zhang N, Hoffman RM, Zhao M. Determination of the optimal route of administration of *Salmonella typhimurium* A1-R to target breast cancer in nude mice. *Anticancer Res* 2012; 32:2501-8; PMID:22753706
- [26] Zhang Y, Miwa S, Zhang N, Hoffman RM, Zhao M. Tumor-targeting *Salmonella typhimurium* A1-R arrests growth of breast-cancer brain metastasis. *Oncotarget* 2015; 6:2615-22; PMID:25575815; <https://doi.org/10.18632/oncotarget.2811>
- [27] Uchugonova A, Zhao M, Zhang Y, Weinigel M, König K, Hoffman RM. Cancer-cell killing by engineered *Salmonella* imaged by multi-photon tomography in live mice. *Anticancer Res* 2012; 32:4331-9; PMID:23060555
- [28] Liu F, Zhang L, Hoffman RM, Zhao M. Vessel destruction by tumor-targeting *Salmonella typhimurium* A1-R is enhanced by high tumor vascularity. *Cell Cycle* 2010; 9:4518-24; PMID:21135579; <https://doi.org/10.4161/cc.9.22.13744>
- [29] Nagakura C, Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Bouvet M, Hoffman RM. Efficacy of a genetically-modified *Salmonella typhimurium* in an orthotopic human pancreatic cancer in nude mice. *Anticancer Res* 2009; 29:1873-8; PMID:19528442
- [30] Yam C, Zhao M, Hayashi K, Ma H, Kishimoto H, McElroy M, Bouvet M, Hoffman RM. Monotherapy with a tumor-targeting mutant of *S. typhimurium* inhibits liver metastasis in a mouse model of pancreatic cancer. *J Surg Res* 2010; 164:248-55; PMID:19766244; <https://doi.org/10.1016/j.jss.2009.02.023>
- [31] Hiroshima Y, Zhao M, Zhang Y, Maawy A, Hassanein MK, Uehara F, Miwa S, Yano S, Momiyama M, Suetsugu A, et al. Comparison of efficacy of *Salmonella typhimurium* A1-R and chemotherapy on stem-like and non-stem human pancreatic cancer cells. *Cell Cycle* 2013; 12:2774-80; PMID:23966167; <https://doi.org/10.4161/cc.25872>
- [32] Hiroshima Y, Zhao M, Maawy A, Zhang Y, Katz MH, Fleming JB, Uehara F, Miwa S, Yano S, Momiyama M, et al. Efficacy of *Salmonella typhimurium* A1-R versus chemotherapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX). *J Cell Biochem* 2014; 115:1254-61; PMID:24435915; <https://doi.org/10.1002/jcb.24769>
- [33] Matsumoto Y, Miwa S, Zhang Y, Hiroshima Y, Yano S, Uehara F, Yamamoto M, Toneri M, Bouvet M, Matsubara H, et al. Efficacy of tumor-targeting *Salmonella typhimurium* A1-R on nude mouse models of metastatic and disseminated human ovarian cancer. *J Cell Biochem*. 2014; 115:1996-2003; PMID:24924355
- [34] Matsumoto Y, Miwa S, Zhang Y, Zhao M, Yano S, Uehara F, Yamamoto M, Hiroshima Y, Toneri M, Bouvet M, et al. Intraperitoneal administration of tumor-targeting *Salmonella typhimurium* A1-R inhibits disseminated human ovarian cancer and extends survival in nude mice. *Oncotarget* 2015; 6:11369-77; PMID:25957417; <https://doi.org/10.18632/oncotarget.3607>
- [35] Yano S, Zhang Y, Zhao M, Hiroshima Y, Miwa S, Uehara F, Kishimoto H, Tazawa H, Bouvet M, Fujiwara T, et al. Tumor-targeting *Salmonella typhimurium* A1-R decoys quiescent cancer cells to cycle as visualized by FUCCI imaging and become sensitive to chemotherapy. *Cell Cycle* 2014; 13:3958-63; PMID:25483077; <https://doi.org/10.4161/15384101.2014.964115>
- [36] Hiroshima Y, Zhang Y, Zhao M, Zhang N, Murakami T, Maawy A, Mii S, Uehara F, Yamamoto M, Miwa S, et al. Tumor-targeting *Salmonella typhimurium* A1-R in combination with Trastuzumab eradicates HER-2-positive cervical cancer cells in patient-derived mouse models. *PLoS One* 2015; 10:e0120358; PMID:26047477; <https://doi.org/10.1371/journal.pone.0120358>
- [37] Murakami T, Igarashi K, Kawaguchi K, Kiyuna T, Zhang Y, Zhao M, Hiroshima Y, Nelson SD, Dry SM, Li Y, et al. Tumor-targeting *Salmonella typhimurium* A1-R regresses an osteosarcoma in a patient-derived xenograft model resistant to a molecular-targeting drug. *Oncotarget* 2017; 8:8035-42; PMID:28030831
- [38] Kawaguchi K, Murakami T, Suetsugu A, Kiyuna T, Igarashi K, Hiroshima Y, Zhao M, Zhang Y, Bouvet M, Clary B, et al. High-efficacy targeting of colon-cancer liver metastasis with *Salmonella typhimurium* A1-R via intra-portal-vein injection in orthotopic nude-mouse models. *Oncotarget* 2017; 8:19065-73.
- [39] Blagosklonny MV. Matching targets for selective cancer therapy. *Drug Discov Today* 2003; 8:1104-7; PMID:14678733; [https://doi.org/10.1016/S1359-6446\(03\)02806-X](https://doi.org/10.1016/S1359-6446(03)02806-X)
- [40] Blagosklonny MV. Teratogens as anti-cancer drugs. *Cell Cycle* 2005; 4:1518-21; PMID:16258270; <https://doi.org/10.4161/cc.4.11.2208>
- [41] Blagosklonny MV. Treatment with inhibitors of caspases, that are substrates of drug transporters, selectively permits chemotherapy-induced apoptosis in multidrug-resistant cells but protects normal cells. *Leukemia* 2001; 15:936-41; PMID:11417480; <https://doi.org/10.1038/sj.leu.2402127>
- [42] Blagosklonny MV. Target for cancer therapy: proliferating cells or stem cells. *Leukemia* 2006; 20:385-91; PMID:16357832; <https://doi.org/10.1038/sj.leu.2404075>

- [43] Apontes P, Leontieva OV, Demidenko ZN, Li F, Blagosklonny MV. Exploring long-term protection of normal human fibroblasts and epithelial cells from chemotherapy in cell culture. *Oncotarget* 2011; 2:222-33; PMID:21447859; <https://doi.org/10.18632/oncotarget.248>
- [44] Blagosklonny MV. Tissue-selective therapy of cancer. *Br J Cancer* 2003; 89:1147-51; PMID:14520435; <https://doi.org/10.1038/sj.bjc.6601256>
- [45] Uchugonova A, Duong J, Zhang N, König K, Hoffman RM. The bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle. *J Cell Biochem* 2011; 112:2046-50; PMID:21465525; <https://doi.org/10.1002/jcb.23122>
- [46] Kawaguchi K, Igarashi K, Murakami T, Chmielowski B, Kiyuna T, Zhao M, Zhang Y, Singh A, Unno M, Nelson SD, et al. Tumor-targeting *Salmonella typhimurium* A1-R combined with temozolomide regresses malignant melanoma with a BRAF-V600E mutation in a patient-derived orthotopic xenograft (PDOX) model. *Oncotarget* 2016; 7(52):85929-36