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Authors

Murakami, Takashi
Kiyuna, Tasuku
Kawaguchi, Kei
et al.

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REPORT

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

Takashi Murakami[a,b,c], Tasuku Kiyuna[a], Kei Kawaguchi[a], Kentaro Igarashi[a], Arun S. Singh[d], Yukihiko Hiroshima[c], Yong Zhang[a], Ming Zhao[a], Kentaro Miyake [a,b,c], Scott D. Nelson[e], Sarah M. Dry[e], Yunfeng Li[e], Jonathan C. DeLong[b], Thinzar M. Lwin[b], Takashi Chishima[c], Kuniya Tanaka[c], Michael Bouvet[b], Itaru Endo [c], Fritz C. Eilber [f], and Robert M. Hoffman[a,b]

[a]AntiCancer, Inc., San Diego, CA, USA; [b]Department of Surgery, University of California, San Diego, CA, USA; [c]Department of Gastroenterological Surgery, Graduate School of Medicine, Yokohama City University, Yokohama, Japan; [d]Division of Hematology-Oncology, University of California, Los Angeles, CA, USA; [e]Department of Pathology, University of California, Los Angeles, CA, USA; [f]Division of Surgical Oncology, University of California, Los Angeles, CA, USA

ABSTRACT

William B. Coley developed bacterial therapy of cancer more than 100 years ago and had clinical success. James Ewing, a very famous cancer pathologist for whom the Ewing sarcoma is named, was Coley's boss at Memorial Hospital in New York and terminated Coley's bacterial therapy of cancer. A tumor from a patient with soft-tissue Ewing's sarcoma, who failed doxorubicin (DOX) therapy, was previously implanted in nude mice to establish a patient-derived orthotopic xenograft (PDOX) model. In the present study, the Ewing's sarcoma PDOX was treated with tumor-targeting *S. typhimurium* A1-R expressing green fluorescent (GFP), alone and in combination with DOX. *S. typhimurium* A1-R-GFP was detected in the tumors after intratumor (i.t.) or intravenous (i.v.) injection. The combination of *S. typhimurium* A1-R and DOX significantly reduced tumor weight (37.8 ± 15.6 mg) compared to the untreated control (73.8 ± 10.1 mg, $P < 0.01$). *S. typhimurium* A1-R monotherapy-treated tumors tended to be smaller (50.9 ± 17.8 mg, $P = 0.051$). DOX monotherapy did not show efficacy (66.3 ± 26.4 mg, $P = 0.82$), as was the case with the patient. The PDOX model faithfully replicated the DOX resistance the Ewing's sarcoma had in the patient. *S. typhimurium* A1-R converted the Ewing's sarcoma from DOX resistant to sensitive. One can only wonder how bacterial therapy and immunotherapy of cancer would have developed over the past 80 years if Ewing did not stop Coley.

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Introduction

Ewing's sarcoma is a recalcitrant malignancy that usually occurs in adolescence and young adulthood. Multimodal treatment for localized Ewing's sarcoma has improved the 5-year survival to 65–70%. However, patients with metastatic disease have a 30% of 5-year survival rate.[1] Therefore, development of effective treatment for Ewing's sarcoma is needed.

William B. Coley developed bacterial therapy of cancer more than 100-years ago and had clinical success.[2,3]

James Ewing was a very famous cancer pathologist for whom the Ewing sarcoma is named, was a bitter opponent of Coley's work at Memorial Hospital in New York and finally ended Coley's career and bacterial therapy of cancer.

In the current century, bacterial therapy is making a strong come back to enhance immunotherapy of cancer.[3] The tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R), developed by our laboratory[4,5] is auxotrophic for Leu-Arg, which prevents it from mounting a continuous infection in normal tissues. *S. typhimurium* A1-R was effective against primary and metastatic tumors as monotherapy in nude mouse models of major cancers,[3] including prostate,[4,6] breast,[7-9] lung,[10,11] pancreatic,[12-16] ovarian,[17-18] stomach,[19] and cervical cancer,[20] as well as sarcoma cell lines[21-24] and glioma,[3,25] all of which are highly aggressive tumor models. In addition, *S. typhimurium* A1-R was effective against patient-derived orthotopic models of pancreatic cancer,[15,16] sarcoma[24,26-29] and melanoma.[30-32]

A tumor from a patient with soft-tissue Ewing's sarcoma was previously established as a patient-derived orthotopic xenograft (PDOX) model.[28] In the present study, we observed that *S. typhimurium* A1-R converted the Ewing's sarcoma from doxorubicin (DOX)-resistant to DOX-sensitive.

Results and discussion

Ewing's sarcoma PDOX tumor recapitulates the original patient tumor

Four weeks after orthotopic implantation, the Ewing's sarcoma PDOX tumor grew to more than 60 mm^3 in the right chest wall

CONTACT Fritz C. Eilber, MD fceilber@mednet.ucla.edu 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, USA; Robert M. Hoffman, PhD all@anticancer.com AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111, USA.

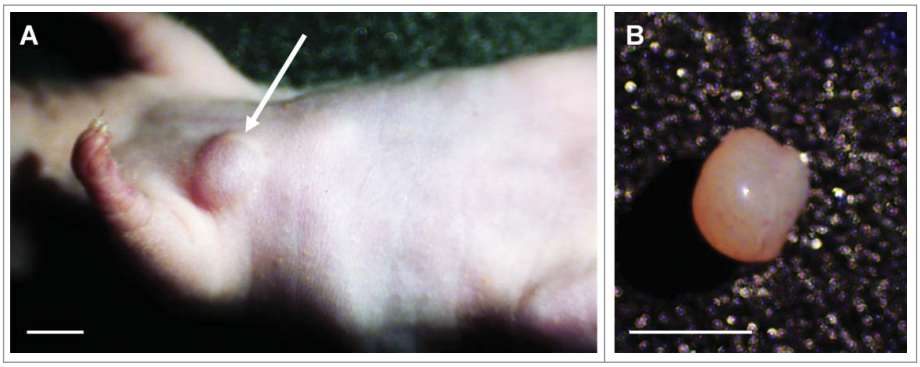

Figure 1. Established Ewing's sarcoma PDOX model. (A) Four weeks after tumor implantation in the space between the pectoral muscle and intercostal muscle, the Ewing's sarcoma PDOX tumors grew in the chest wall (arrow). (B) Resected tumor from the same mouse. Scale bars: 5 mm.

of nude mice (Fig. 1). Histologically, the patient's tumor demonstrated an infiltrative proliferation of small round blue cells. Cancer cells showed round-to-ovoid hyperchromatic nuclei, and scanty eosinophilic-to-clear cytoplasm (Fig. 2A). Histology of the PDOX tumor had a similar morphologic appearance to the original patient tumor (Fig. 2B).

S. typhimurium *A1-R-GFP was detected in treated PDOX tumors*

GFP-expressing *S. typhimurium* A1-R was detected in tumors on the next day after the 2nd i.t. injection (Fig. 3A, B) or i.v. injection of *S. typhimurium* A1-R-GFP (Fig. 3C, D). More *S. typhimurium* A1-R-GFP was detected in the tumor after i.t. injection than i.v. injection, perhaps because the bacteria were directly injected. However, even with i.v. injection, a large amount of bacteria targeted the tumor from the circulation.

S. typhimurium *A1-R (i.t. and i.v.) inhibited tumor growth in the Ewing's sarcoma PDOX*

The tumor doubling time of the Ewing's sarcoma PDOX was 12.7 days. Both administrative routes (i.t. and i.v.) of *S. typhimurium* A1-R inhibited tumor growth (Fig. 4). A PDOX was previously established in the right chest wall of nude mice which corresponded to the origin of the tumor in the patient. Eighteen PDOX mice were randomized into three groups when tumor volume reached 60 mm^3: untreated control; *S. typhimurium* A1-R intratumor administration (i.t., 5×10^7 CFU/25 μl, day 1 and 8), and *S. typhimurium* A1-R intravenous administration (i.v., 5×10^7 CFU/100 μl, day 1 and 8). Tumor growth was significantly suppressed in both of the *S. typhimurium*-treated groups. However, *S. typhimurium* A1-R i.v. was more effective than i.t. injection.

S. typhimurium *A1-R i.v. increased the efficacy of doxorubicin in the Ewing's sarcoma PDOX model*

S. typhimurium A1-R i.v. in combination with DOX, was then tested. Twenty PDOX mice were prepared as described above and randomized into the following groups; G1, untreated control; G2, DOX (i.v., 3 mg/kg, day 1 and 8); G3, *S. typhimurium* A1-R (i.v., 5×10^7 CFU/100 μl, day 1 and 8); G4, combination of *S. typhimurium* A1-R (i.v., 5×10^7 CFU/100 μl, day 1 and 8); and DOX (i.v., 3 mg/kg, day 4 and 11).

Only the combination of *S. typhimurium* A1-R and DOX significantly reduced tumor weight (37.8 μ $\pm$ 15.6 mg) compared to the untreated control (73.8 $\pm$ 10.1 mg, $P < 0.01$). *S. typhimurium* A1-R monotherapy-treated tumors tended to be smaller (50.9 $\pm$ 17.8 mg, $P = 0.051$). DOX monotherapy did not show efficacy (66.3 $\pm$ 26.4 mg, $P = 0.82$), as was the case with the patient (Fig. 5). Mouse body

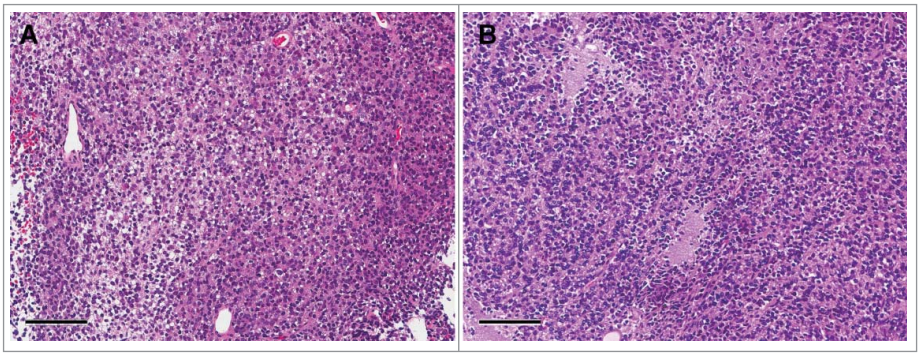

Figure 2. Histological comparison between patient original tumors and a PDOX tumor. (A) H&E staining of the patient original tumor and (B) untreated PDOX tumor. Scale bars: 100 μm.

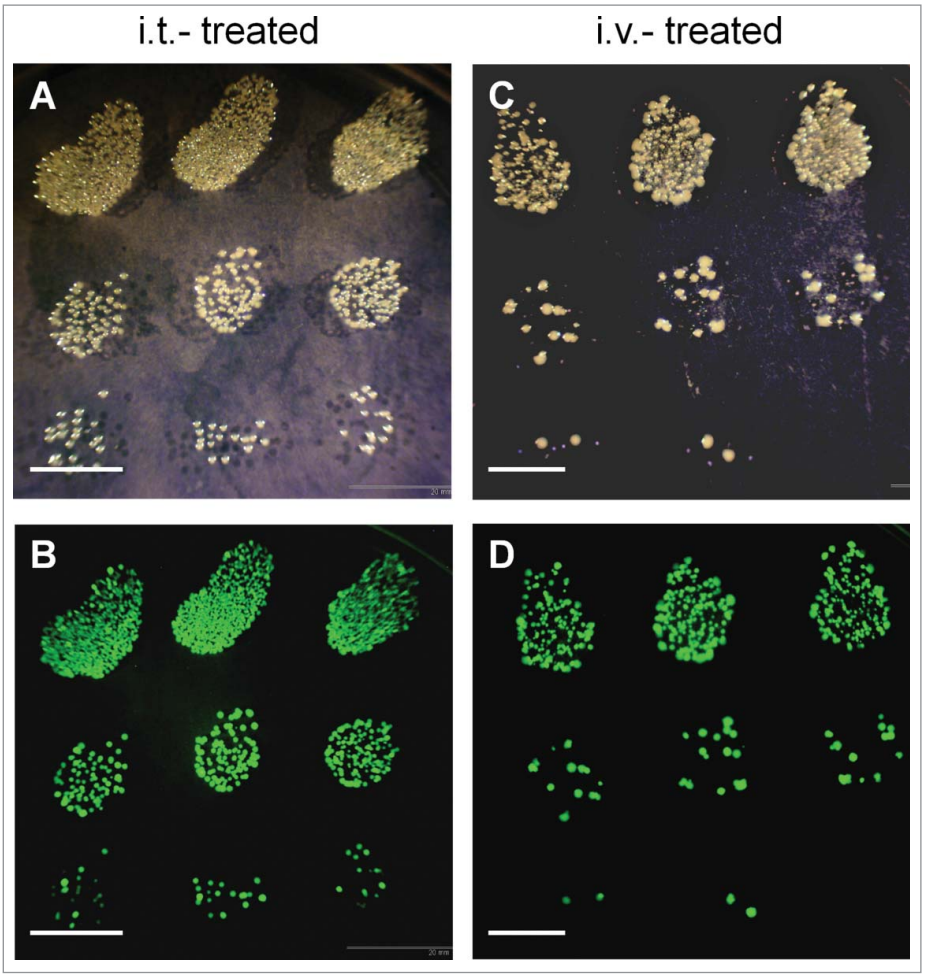

Figure 3. Bacterial culture from *S. typhimurium* A1-R-treated Ewing's sarcoma PDOX. Bright field (A, C) and fluorescence (B, D) images of GFP-expressing *S. typhimurium* A1-R colonies cultured in LB-agar. Administered *S. typhimurium* either by i.t. (A, B) or i.v. (C, D) was detected in treated tumors 24 hours after the second injection of *S. typhimurium* A1-R (day 9). Scale bars: 10 mm. GFP, green fluorescent protein. OV100 imaging.

weight was not significantly different between groups at any time point.

Bacterial therapy has potential side effects, even though they were not observed in the present study. Such side effects could include infection outside the tumor and potential unwanted immunological reaction.

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.[33-38]

The aim of the present study was to develop *S. typhimurium* A1-R treatment for an Ewing's sarcoma using different administrative routes and in combination with standard first-line DOX.

Conclusions

The present study has demonstrated the potential of *S. typhimurium* A1-R to be an effective treatment as both monotherapy and combination with first-line therapy for Ewing's sarcoma. Ironically, bacterial therapy was highly effective on a PDOX model of Ewing's sarcoma, demonstrating that the very treatment that was halted by Ewing was found more than 80 years later to be highly effective on the sarcoma named for him. One can only wonder how bacterial therapy and immunotherapy of cancer would have developed over the past 80 years if Ewing did not stop Coley.

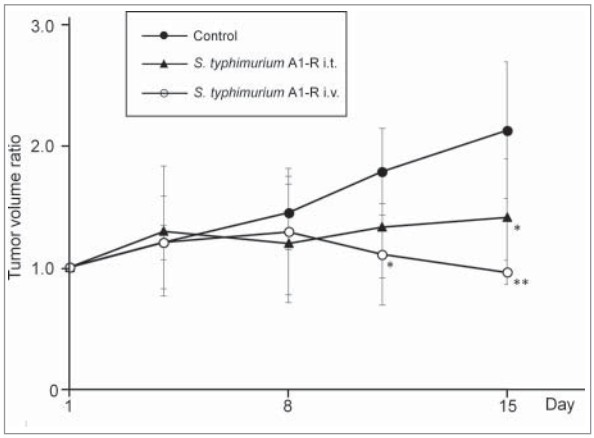

Figure 4. *S. typhimurium* A1-R administered i.v. or i.t. on the Ewing's sarcoma PDOX Line graph shows tumor volume ratio. *S. typhimurium* A1-R was administered i.v. or i.t. Please see the Materials and Methods for doses and schedules. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to control. Error bars: ± 1 SD. i.t., intratumoral injection; i.v., intravenous injection.

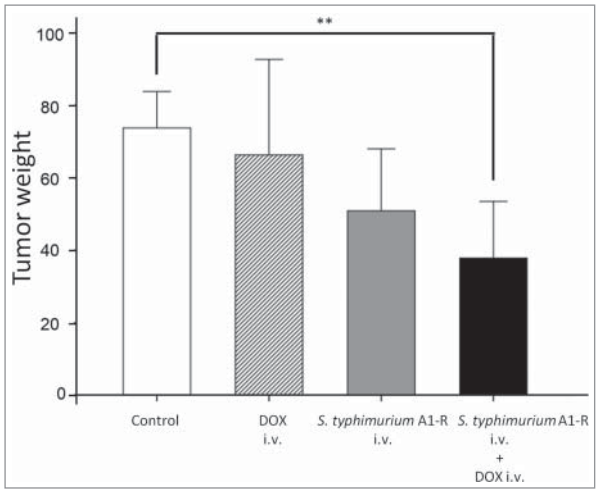

Figure 5. Combination therapy of intravenous *S. typhimurium* A1-R (i.v.) combined with doxorubicin in the Ewing's sarcoma PDOX model. Bar graphs show resected tumor weight in each group. Please see the Materials and Methods for doses and schedules.

Materials and methods

Mice

Athymic *nu/nu* nude mice (AntiCancer Inc., San Diego, CA), 4–6 weeks old, were used in this study under Assurance Number A3873-1. The animals were fed an autoclaved laboratory rodent diet.[28] Animals were anesthetized by subcutaneous injection of a 0.02 ml solution of 25 mg/kg ketamine, 19 mg/kg xylazine, and 0.60 mg/kg acepromazine maleate. Animals were housed with no more than 5 per cage and housed in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under standard conditions of 12-hour light/dark cycles.

Patient-derived Ewing's sarcoma tumor

The female 46-year-old patient was previously diagnosed with Ewing's sarcoma in the right chest wall and received neoadjuvant chemotherapy including DOX. Then the tumor was resected by JY in the Department of Surgery, University of California, Los Angeles (UCLA). Written informed consent was obtained from the patient, and the Institutional Review Board (IRB) of UCLA approved this experiment.[28]

Establishment of a PDOX model of Ewing's sarcoma

Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI), including pancreatic,[16,39-41] breast,[42] ovarian,[43] lung,[44] cervical,[45] colon,[46-48] stomach,[49] sarcoma,[26-28,50] and melanoma.[30-32]

A Ewing's sarcoma PDOX was previously established on the right chest wall where a single tumor fragment was implanted orthotopically into the layer between pectoral muscle and intercostal muscle in nude mice.[28]

Preparation of S. typhimurium A1-R

GFP-expressing *S. typhimurium* A1-R bacteria (AntiCancer Inc.,) were grown overnight on 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.[4,6,7]

Efficacy of S. typhimurium A1-R i.v. vs. i.t. on the Ewing's sarcoma PDOX

PDOX tumors (n = 18) were divided into 3 groups when tumor volume reached 60 mm^3: untreated control (n = 6); *S. typhimurium* A1-R (n = 6, i.t., 5×10^7 CFU/25 μl, day 1 and 8); *S. typhimurium* A1-R (n = 6, i.v., 5×10^7 CFU/100 μl, day 1 and 8). The mice were followed up until day 15. Tumor length, width, and mouse body weight were measured twice a week. Tumor volume was calculated by the following formula: Tumor volume (mm^3) = length (mm) × width (mm) × width (mm) × 1/2. Tumor volume ratio was defined as the ratio of volume on each measurement day relative to day 0.

Efficacy of S. typhimurium A1-R in combination with DOX on the Ewing's sarcoma PDOX

PDOX tumors (n = 20) were divided into following 4 groups when the tumor volume reached 60 mm^3: G1, untreated control (n = 5); G2; DOX (n = 5; i.v., 3 mg/kg, day 1 and 8); G3, *S. typhimurium* A1-R (n = 5, i.v., 5×10^7 CFU/100 μl, day 1 and 8); and G4; combination of *S. typhimurium* A1-R (n = 5, i.v., 5×10^7 CFU/100 μl, day 1 and 8) and DOX (n = 5, i.v., 3 mg/kg, day 4 and 11). Mice were sacrificed and tumors were removed on day 15 for weight determination. Tumor growth measurement and evaluation was performed as described above.

Histological analysis

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (5 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 (Olympus Corp., Tokyo, Japan). Images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada).[28]

Bacterial culture from tumor tissue

To identify *S. typhimurium* A1-R in treated tumors, two Ewing's sarcoma PDOX mice were treated with *S. typhimurium* A1-R i.t. or i.v. These mice were sacrificed and tumors were resected on day 9 to culture bacteria. After removal of tumors from PDOX mice, the tumor specimens were homogenized and suspended in PBS (phosphate-buffered saline, Corning, New York, NY). The suspension was serially diluted, then cultured in LB agar for 12 hours. GFP-expressing colonies of *S. typhimurium* A1-R were detected with the OV100 Small Animal Imaging System (Olympus, Tokyo, Japan).[51]

Statistical analysis

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

Dedication

This paper is dedicated to the memory of A. R. Moossa, MD and Sun Lee, MD.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Kentaro Miyake http://orcid.org/0000-0002-4680-4317
Itaru Endo http://orcid.org/0000-0001-5520-8114
Fritz C. Eilber http://orcid.org/0000-0003-3336-9333

References

[1] Gaspar N, Hawkins DS, Dirksen U, Lewis IJ, Ferrari S, Le Deley MC, Kovar H, Grimer R, Whelan J, Claude L, et al. Ewing Sarcoma: Current management and future approaches through collaboration. J Clin Oncol 2015; 33:3036-3046; PMID:26304893; https://doi.org/10.1200/JCO.2014.59.5256

[2] Nauts HC, Fowler GA, Bogatko FH. A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man; a critical analysis of 30 inoperable cases treated by Coley's mixed toxins, in which diagnosis was confirmed by microscopic examination selected for special study. Acta Med Scand Suppl 1953; 276:1-103.

[3] Hoffman RM, editor. Bacterial Therapy of Cancer: Methods and Protocols. Methods in Molecular Biology 1409. Walker, John M., series ed. New York: Humana Press (Springer Science+Business Media); 2016.

[4] Zhao M, Yang M, Li XM, 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

[5] Zhang Y, Zhang N, Zhao M, Hoffman RM. Comparison of the selective targeting efficacy of *Salmonella typhimurium* A1-R and VNP20009 on the Lewis lung carcinoma in nude mice. Oncotarget 2015; 6:14625-31; PMID:25714030; https://doi.org/10.18632/oncotarget.3342

[6] 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; 104:10170-74; PMID:17548809; https://doi.org/10.1073/pnas.0703867104

[7] 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

[8] 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; 32:2501-08; PMID:22753706

[9] 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

[10] Uchugonova A, Zhao M, Zhang Y, Weinigel M, König K, Hoffman RM. Cancer-cell killing by engineered Salmonella imaged by multiphoton tomography in live mice. Anticancer Res 2012; 32:4331-7; PMID:23060555

[11] 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

[12] 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

[13] 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

[14] 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

[15] 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

[16] 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 xenograph (PDOX) and cell line mouse models. Oncotarget 2014; 5:12346-357; PMID:25402324; https://doi.org/10.18632/oncotarget.2641

[17] 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

[18] 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

[19] 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

[20] 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

[21] Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Hoffman RM. Cancer metastasis directly eradicated by targeted therapy with a modified *Salmonella typhimurium*. J Cell Biochem 2009; 106:992-998; PMID:19199339; https://doi.org/10.1002/jcb.22078

[22] Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Kishimoto H, Bouvet M, Hoffman RM. Systemic targeting of primary bone tumor and lung metastasis of high-grade osteosarcoma in nude mice with a tumor-selective strain of *Salmonella typhimurium*. Cell Cycle 2009; 8:870-5; PMID:19221501; https://doi.org/10.4161/cc.8.6.7891

[23] Miwa S, Zhang Y, Baek K-E, Uehara F, Yano S, Yamamoto M, Hiroshima Y, Matsumoto Y, Kimura H, Hayashi K, et al. Inhibition of spontaneous and experimental lung metastasis of soft-tissue sarcoma by tumor-targeting *Salmonella typhimurium* A1-R. Oncotarget 2014; 5:12849-61; PMID:25528763; https://doi.org/10.18632/oncotarget.2561

[24] 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

[25] Momiyama M, Zhao M, Kimura H, Tran B, Chishima T, Bouvet M, Endo I, Hoffman RM. Inhibition and eradication of human glioma with tumor-targeting *Salmonella typhimurium* in an orthotopic nude-mouse model. Cell Cycle 2012; 11:628-32; PMID:22274398; https://doi.org/10.4161/cc.11.3.19116

[26] Hiroshima Y, Zhao M, Zhang Y, Zhang N, Maawy A, Murakami T, Mii S, Uehara F, Yamamoto M, Miwa S, et al. Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice. PLoS One 2015; 10:e0134324; PMID:26237416; https://doi.org/10.1371/journal.pone.0134324

[27] 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 dactolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model. Oncotarget 2016; 7:33046-54; PMID:27105519

[28] 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

[29] Kiyuna T, Murakami T, Tome Y, Igarashi K, Kawaguchi K, Russell T, Eckhardt MA, Crompton J, Singh A, Bernthal N, et al. Labeling the stroma of a patient-derived orthotopic xenograft (PDOX) mouse models of undifferentiated pleomorphic soft-tissue sarcoma with red fluorescent protein for rpaid non-invasive drug screening. J Cell Biochem 2017; 118:361-65; PMID:27357060; https://doi.org/10.1002/jcb.25643

[30] 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

[31] 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

[32] Kawaguchi K, Igarashi K, Murakami T, Chmiewloski 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

[33] Blagosklonny MV. Matching targets for selective cancer therapy. Drug Discov Today 2003; 8:1104-07; PMID:14678733; https://doi.org/10.1016/S1359-6446(03)02806-X

[34] Blagosklonny MV. Teratogens as anti-cancer drugs. Cell Cycle 2005; 4:1518-21; PMID:16258270; https://doi.org/10.4161/cc.4.11.2208

[35] Blagosklonny MV. Treatment with inhibitors of caspases, that are substrates of drug ransporters, 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

[36] 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

[37] 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

[38] Blagosklonny MV. Tissue-selective therapy of cancer. Br J Cancer 2003; 89:1147-51; PMID:14520435; https://doi.org/10.1038/sj.bjc.6601256

[39] 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; 89:5645-49; PMID:1608975; https://doi.org/10.1073/pnas.89.12.5645

[40] 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

[41] 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 xenograph (PDOX) nude-mouse model of human pancreatic cancer. J Surg Oncol 2015; 111:311-5; PMID:25394368; https://doi.org/10.1002/jso.23816

[42] Fu X, Le P, Hoffman RM. A metastatic-orthotopic transplant nude-mouse model of human patient breast cancer. Anticancer Res 1993; 13:901-04; PMID:8352558

[43] 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

[44] 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

[45] 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 xenograph (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

[46] 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

[47] 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

[48] 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; 24:241-47; PMID:24494971; https://doi.org/10.1089/lap.2013.0418

[49] Furukawa T, Kubota T, Watanabe M, Kitajima M, Fu X, 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

[50] 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

[51] Yamauchi K, Yang M, Jiang P, Xu M, Yamamoto N, Tsuchiya H, Tomita K, Moossa AR, Bouvet M, Hoffman RM. Development of real-time subcellular dynamic multicolor imaging of cancer-cell trafficking in live mice with a variable-magnification whole-mouse imaging system. Cancer Res 2006; 66:4208-14; PMID:16618743; https://doi.org/10.1158/0008-5472.CAN-05-3927