ARTICLE
Journal of Cellular Biochemistry 118:2314–2319 (2017)

Journal of Cellular Biochemistry

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

Kei Kawaguchi,[1,2,3] Kentaro Igarashi,[1,2] Takashi Murakami,[1,2] Ming Zhao,[1] Yong Zhang,[1] Bartosz Chmielowski,[4] Tasuku Kiyuna,[1,2] Scott D. Nelson,[5] Tara A. Russell,[6] Sarah M. Dry,[5] Yunfeng Li,[5] Michiaki Unno,[3] Fritz C. Eilber,[6,7]** and Robert M. Hoffman[1,2,7]*

[1]*AntiCancer, Inc., San Diego, California*
[2]*Department of Surgery, University of California, San Diego, California*
[3]*Department of Surgery, Graduate School of Medicine, Tohoku University, Sendai, Japan*
[4]*Division of Hematology–Oncology, University of California, Los Angeles, California*
[5]*Department of Pathology, University of California, Los Angeles, California*
[6]*Division of Surgical Oncology, University of California, Los Angeles, California*
[7]*PDOX Inc., San Diego, California*

ABSTRACT

Previously, a BRAF-V600E-mutant melanoma obtained from the right chest wall of a patient was grown orthotopically in the right chest wall of nude mice to establish a patient-derived orthotopic xenograft (PDOX) model. Trametinib (TRA), an MEK inhibitor, caused tumor regression. In contrast, another MEK inhibitor, cobimetinib (COB) could slow but not arrest growth or cause regression of the melanoma PDOX. First-line therapy temozolomide (TEM) could slow but not arrest tumor growth or cause regression. In addition, vemurafenib (VEM) was not effective even though VEM is supposed to target the BRAF-V600E mutation. We also previously demonstrated that tumor-targeting with *S. typhimurium* A1-R combined with TEM was significantly more effective than either *S. typhimurium* A1-R alone or TEM alone on the melanoma PDOX with the BRAF-V600E mutation. The present study used this PDOX model of melanoma to test its sensitivity to VEM combined with *S. typhimurium* A1-R compared to VEM alone and VEM combined with COB. VEM combined with *S. typhimurium* A1-R was significantly more effective than VEM alone or VEM combined with COB ($P = 0.0216$) which is currently first line therapy for advanced melanoma with a BRAF-V600E mutation. J. Cell. Biochem. 118: 2314–2319, 2017.

KEY WORDS: MELANOMA; PDOX; NUDE MICE; ORTHOTOPIC; DRUG-RESPONSE; VEMURAFENIB; COBIMETINIB; *Salmonella typhimurium* A1-R; TUMOR REGRESSION; PRECISION MEDICINE; COMBINATION THERAPY

Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model in the early 1990s with the technique of surgical orthotopic implantation (SOI) [Hoffman, 2015]. PDOX models were established from patients with colon [Fu et al., 1991; Hiroshima et al., 2014a; Metildi et al., 2014], pancreatic [Fu et al., 1992; Hiroshima et al., 2014b,c, 2015a], breast [Fu and Hoffman, 1993], ovarian [Fu et al., 1993], lung [Wang et al., 1992], and stomach cancer [Furukawa et al., 1993], and mesothelioma [Astoul et al., 1996] resulting in primary and metastatic tumor growth very similar to that of the patient [Furukawa et al., 1993]. Recently, PDOX models of sarcoma have been developed [Hiroshima et al., 2015b,c; Kiyuna et al., 2016;

*Correspondence to: Robert M. Hoffman, PhD, AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111. E-mail: all@anticancer.com
**Correspondence to: Prof. Fritz C. Eilber, MD, Division of Surgical Oncology, 10833 LeConte Avenue, Rm 54-140 CHS, Los Angeles, CA 90095-1782. E-mail: fceilber@mednet.ucla.edu
Manuscript Received: 13 January 2017; Manuscript Accepted: 18 January 2017
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 20 January 2017
DOI 10.1002/jcb.25886 • © 2017 Wiley Periodicals, Inc.

Murakami et al., 2016a,b], cervical cancer [Hiroshima et al., 2015d] as well as melanoma [Kawaguchi et al., 2016a,b; Yamamoto et al., 2016].

Metastatic melanoma is a recalcitrant cancer with a survival rate below 10% [Hauschild et al., 2012]. Recently-developed immunotherapy for melanoma has not significantly increased the 5-year survival rate [Gandini et al., 2016]. The chemotherapy drugs, dacarbazine, and cisplatinum have had limited efficacy on melanoma [Rabik and Dolan, 2007; Tang et al., 2016]. Vemurafenib (VEM), a tyrosine kinase inhibitor targets BRAF-V600E kinase, has had efficacy on BRAF-V600E-mutant melanoma [Chapman et al., 2011; Larkin et al., 2014; McArthur et al., 2014; Sosman et al., 2012]. VEM combined with cobimetinib (COB), another MEK inhibitor, has also had some efficacy on advanced melanoma with a BRAF-V600E mutation. However, more effective approaches to melanoma treatment are needed.

We previously established a PDOX model of melanoma, with a BRAF-V600E mutation, that was resected from the right chest wall of a patient. The melanoma was grown orthotopically in the right chest wall of nude mice to establish the PDOX model. VEM would be considered to be a strong candidate for BRAF-V600E mutated melanoma as first line therapy; however, VEM was not effective [Kawaguchi et al., 2016a]. Trametinib (TRA), a MEK inhibitor, caused tumor regression. In contrast, COB, another MEK inhibitor, was not effective on the melanoma. Temozolomide (TEM), used for first line therapy of melanoma was also not effective.

A tumor-targeting strain of *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R), was developed by our laboratory [Hoffman and Zhao, 2014; Hoffman, 2016]. *S. typhimurium* A1-R is attenuated by Leu–Arg auxotrophic mutations, which prevents it from continuous infection of normal tissues. *S. typhimurium* A1-R has high efficacy for orthotopic models of major cancer types including prostate [Zhao et al., 2005, 2007], breast [Zhao et al., 2006], lung [Liu et al., 2010; Uchugonova et al., 2012], pancreatic [Hiroshima et al., 2013, 2014c,d; Nagakura et al., 2009; Yam et al., 2010], ovarian [Matsumoto et al., 2014, 2015], stomach [Yano et al., 2014], and cervical cancer [Hiroshima et al., 2015e], as well as sarcoma cell lines [Hayashi et al., 2009a,b; Miwa et al., 2014], and glioma [Kimura et al., 2010; Momiyama et al., 2012], all of which are highly aggressive tumor models. *S. typhimurium* A1-R also has efficacy on PDOX models of pancreatic cancer [Hiroshima et al., 2014a], sarcoma [Hiroshima et al., 2015c; Kiyuna et al., 2016; Murakami et al., 2016a,b] as well as melanoma [Kawaguchi et al., 2016b; Yamamoto et al., 2016].

In another previous study with the PDOX model of the BRAF-V600E mutant melanoma, TEM combined with *S. typhimurium* A1-R was significantly more effective than either *S. typhimurium* A1-R alone or TEM alone [Kawaguchi et al., 2016b].

The combination of *S. typhimurium* A1-R and cisplatinum (CDDP) also significantly suppressed the growth of another melanoma PDOX with less side effects than high-dose CDDP monotherapy [Yamamoto et al., 2016].

In the present study, we show in the PDOX model of VEM-resistant melanoma with a BRAF-V600E mutation, that *S. typhimurium* A1-R combined with VEM was highly effective.

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 efficacy 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 in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. All mouse surgical procedures and imaging were performed with the animals anesthetized by subcutaneous injection of a ketamine mixture (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 CO_2 inhalation if 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.

PATIENT-DERIVED TUMOR

A 75-yaer-old female patient was diagnosed with a melanoma of the right chest wall. The tumor was previously resected in the Department of Surgery, University of California, Los Angeles (UCLA). Written informed consent was provided by the patient, and the Institutional Review Board (IRB) of UCLA approved this experiment [Kawaguchi et al., 2016a,b].

ESTABLISHMENT OF PDOX MODELS OF MELANOMA BY SURGICAL ORTHOTOPIC IMPLANTATION (SOI)

Previously, a fresh sample of the melanoma of the patient was obtained and transported immediately to the laboratory at AntiCancer, Inc., on wet ice. The sample was cut into 5-mm fragments and implanted subcutaneously in nude mice. After 3 weeks, the subcutaneously-implanted tumors grew to more than 10 mm in diameter. The subcutaneously-grown tumors were then harvested and cut into small fragments (3 mm^3). After nude mice were anesthetized with the ketamine solution described above, a 5-mm skin incision was made on the right chest into the chest wall, which was split to make space for the melanoma tissue fragment. A single tumor fragment was implanted orthotopically into the space to establish the PDOX model. The wound was closed with a 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ) [Kawaguchi et al., 2016a,b].

PREPARATION AND ADMINISTRATION 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) and then diluted 1:10 in LB medium. Bacteria were harvested at late-log phase, washed with PBS, and then diluted in PBS [Zhao et al., 2005, 2006, 2007].

TREATMENT STUDY DESIGN IN THE PDOX MODEL OF MELANOMA

PDOX mouse models were randomized into six groups of 10 mice each: untreated control (n = 10); VEM (30 mg/kg, po qd × 14); COB (5 mg/kg, po qd × 14); *S. typhimurium* A1-R (5×10^7 CFU/100 μl, i.v., qw × 2); COB (30 mg/kg, 5 mg/kg, po qd × 14) combined with VEM (30 mg/kg, po qd × 14); VEM (30 mg/kg, po qd × 14) combined with *S. typhimurium* A1-R (5×10^7 CFU/100 μl, i.v., qw × 2). Tumor length and width were measured twice a week. Tumor volume was calculated with the following formula: Tumor volume (mm^3) = length (mm) × width (mm) × width (mm) × 1/2. Data are presented as mean ± SD. The tumor volume ratio is defined at the tumor volume at any given time point relative to the initial tumor volume.

CONFOCAL MICROSCOPY

The FV1000 confocal microscope (Olympus, Tokyo, Japan) was used for high-resolution imaging. Fluorescence images were obtained using the 20×/0.50 UPLAN FLN and 40×/1.3 oil Olympus UPLAN FLN objectives [Uchugonova et al., 2011].

HISTOLOGICAL EXAMINATION

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 protocols. Histological examination was performed with a BHS System Microscope (Olympus Corporation, Tokyo, Japan). Images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada) [Kawaguchi et al., 2016a,b].

STATISTICAL ANALYSIS

JMP version 11.0 was used for all statistical analyses. Significant differences for continuous variables were determined using the Mann–Whitney *U*-test. Line graphs expressed average values and error bar showed SD. A probability value of $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

VEM, *S. typhimurium* A1-R, COB, VEM combined with COB, and VEM combined with *S. typhimurium* A1-R were effective against the BRAF-V600E mutant melanoma PDOX, to varying degrees, compared to the untreated control: VEM, $P = 0.0210$; *S. typhimurium* A1-R, $P = 0.0081$; COB, $P = 0.0001$; VEM combined with COB, $P < 0.0001$; VEM combined with *S. typhimurium* A1-R, $P < 0.0001$. In addition, VEM combined with *S. typhimurium* A1-R was the most effective compared to other therapies (VEM alone: $P = 0.0007$; *S. typhimurium* A1-R alone: $P = 0.0021$; COB alone: $P = 0.0122$; VEM combined with COB: $P = 0.0216$) (Fig. 1). The relative body weight on day 14 compared with day 0 did not significantly differ between each treatment group (Fig. 2).

Confocal microscopy showed that *S. typhimurium* A1-R could directly target the melanoma PDOX (Fig. 3).

The histology of the original patient tumor and the untreated PDOX tumor were similar. The original tumor was slightly melanotic, but the PDOX tumor did not appear to contain melanin [Kawaguchi et al.,

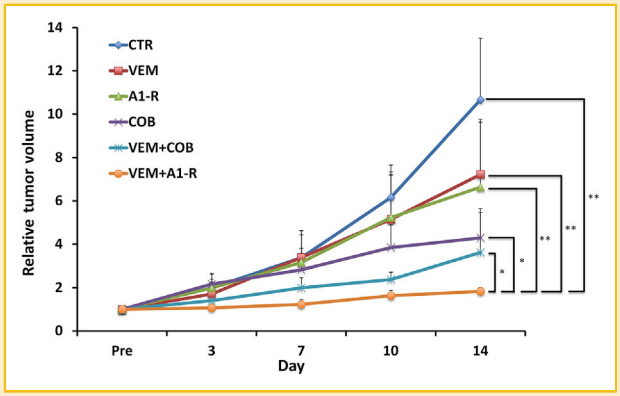

Fig. 1. Tumor growth curves. Line graphs show relative tumor volume at each point relative to the initial tumor volume. Please see Materials and Methods section for drug dose, route, and schedule. $^{**}P < 0.01$. $^{*}P < 0.05$. Error bars: ± SD.

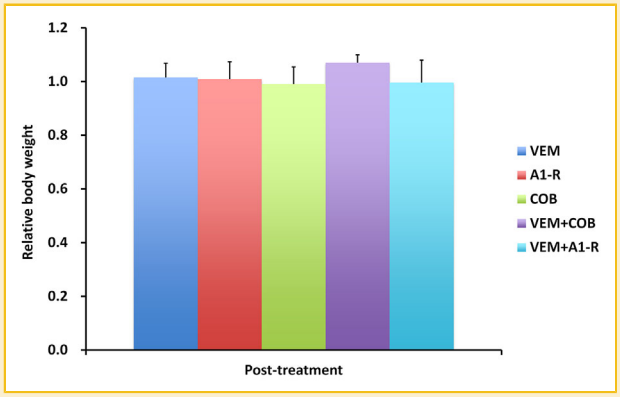

Fig. 2. Effect of treatment on mouse body weight. Bar graphs show relative body weight in each treatment group at post-treatment relative to the initial body weight.

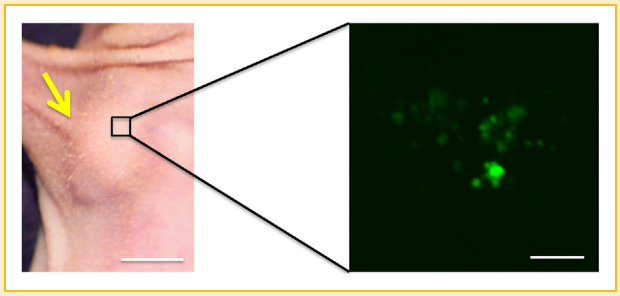

Fig. 3. Fluorescence imaging of *S. typhimurium* A1-R-GFP targeting the melanoma PDOX. Confocal imaging with the FV1000. *S. typhimurim* A1-R is visualized by GFP expression. Bars: left panel: 5 mm, right panel: 12.5 μm.

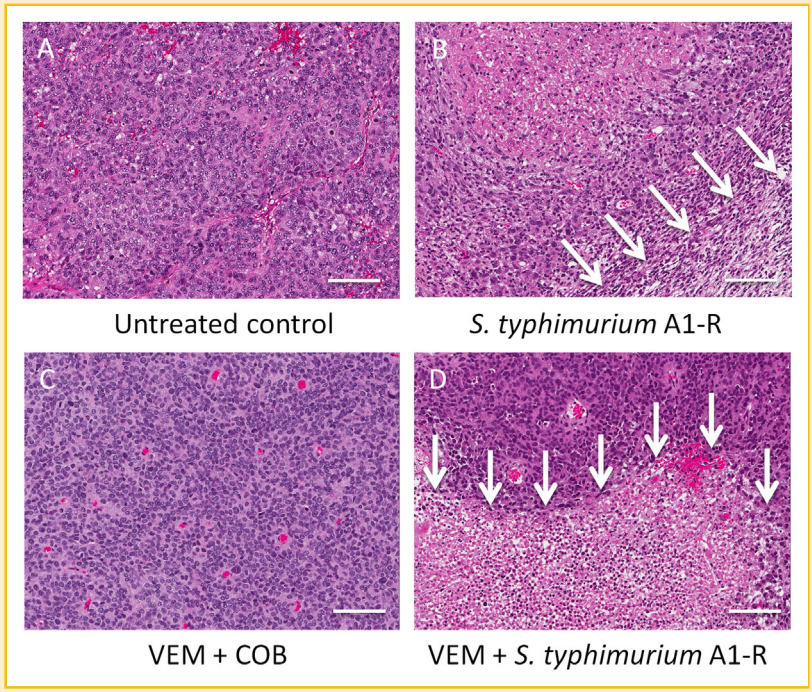

Fig. 4. Tumor histology. (A) Untreated control. (B) Tumor treated with *S. typhimurium* A1-R. (C) Tumor treated with the combination of VEM and COB inhibited. (D) Tumor treated with the combination of VEM and *S. typhimurium* A1-R. White allows: necrotic areas. Scale bars: 100 μm.

2016a,b]. Necrosis was observed in the *S. typhimurium* A1-R treatment group. Necrosis was more extensive in the group treated with VEM combined with *S. typhimurium* A1-R (Fig. 4).

In the present study, the efficacy of VEM alone was significantly less than other therapies (COB ($P = 0.0470$); VEM combined with COB ($P = 0.0163$); and VEM combined with *S. typhimurium* A1-R ($P = 0.0007$), on day 14 (Fig. 1). Thus, despite the BRAF-V600E mutation in the melanoma, the PDOX was relatively resistant to VEM demonstrating that "molecular targeting" may be insufficient for effective therapy.

COB is an MEK inhibitor. The combination of VEM and COB improved progression-free survival (PFS) in melanoma compared to VEM plus placebo in a clinical trial [Larkin et al., 2014]. VEM combined with COB is being used for advanced melanoma with BRAF-V600E mutations [Chapman et al., 2011]. In the present study, VEM combined with COB was also significantly more effective than VEM monotherapy ($P = 0.0163$) (Fig. 1). VEM combined with *S. typhimurium* A1-R was significantly more effective than other therapies, including VEM combined with COB. This is the first report that showed the combination of bacterial therapy with a molecular targeting agent, VEM, is effective in melanoma.

Despite progress in melanoma therapy, there is still no cure for stage III and IV disease due to drug resistance, tumor heterogeneity and an immunosuppressive tumor environment [Brożyna et al., 2016; Chapman et al., 2011; Flaherty et al., 2014; Slominski and Carlson, 2014; Tang et al., 2016]. In addition, the presence of melanin appears to interfere with chemotherapy and radiotherapy of this recalcitrant disease [Brożyna et al., 2016]. The present study demonstrated that *S. typhimurium* A1-R has promising potential to increase the efficacy of a molecular targeting drug against metastatic melanoma.

REFERENCES

Astoul P, Wang X, Colt HG, Boutin C, Hoffman RM. 1996. A patient-like human malignant pleural mesothelioma nude-mouse model. Oncology Reports 3:483–487.

Brożyna AA, Jóźwicki W, Roszkowski K, Filipiak J, Slominski AT. 2016. Melanin content in melanoma metastases affects the outcome of radiotherapy. Oncotarget 7:17844–17853.

Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA; BRIM-3 Study Group. 2011. BRIM-3 Study Group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364:2507–2516.

Flaherty LE, Othus M, Atkins MB, Tuthill RJ, Thompson JA, Vetto JT, Haluska FG, Pappo AS, Sosman JA, Redman BG, Moon J, Ribas A, Kirkwood JM, Sondak VK. 2014. Southwest Oncology Group S0008: A phase III trial of high–dose interferon Alfa–2b versus cisplatin, vinblastine, and dacarbazine, plus interleukin-2 and interferon in patients with high-riskmelanoma-an intergroup study of cancer and leukemia Group B, Children's Oncology Group, Eastern Cooperative Oncology Group, and Southwest Oncology Group. J Clin Oncol 32:3771–3778.

Fu X, Hoffman RM. 1993. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. Anticancer Res 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, Hoffman RM. 1993. 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, Fu X, 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.

Gandini S, Massi D, Mandala M. 2016. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review and meta-analysis. Crit Rev Oncol Hematol 100:88–98.

Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH, Jr., Kaempgen E, Martín-Algarra S, Karaszewska B, Mauch C, Chiarion-Sileni V, Martin AM, Swann S, Haney P, Mirakhur B, Guckert ME, Goodman V, Chapman PB. 2012. Dabrafenib in BRAF-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet 380:358–365.

Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Hoffman RM. 2009a. Cancer metastasis directly eradicated by targeted therapy with a modified *Salmonella typhimurium*. J Cell Biochem 106:992–998.

Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Kishimoto H, Bouvet M, Hoffman RM. 2009b. 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 8:870–875.

Hiroshima Y, Zhao M, Zhang Y, Maawy A, Hassanein MK, Uehara F, Miwa S, Yano S, Momiyama M, Suetsugu A, Chishima T, Tanaka K, Bouvet M, Endo I, Hoffman RM. 2013. Comparison of efficacy of *Salmonella typhimurium* A1-R and chemotherapy on stem-like and non-stem human pancreatic cancer cells. Cell Cycle 12:2774–2780.

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, Endo I, Hoffman RM. 2014a. 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 A, Zhang Y, Murakami T, Momiyama M, Mori R, Matsuyama R, Katz MH, Fleming JB, Chishima T, Tanaka K, Ichikawa Y, Endo I, Hoffman RM, Bouvet M. 2014b. 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, Zhang Y, Murakami T, Maawy AA, Miwa S, Yamamoto M, Yano S, Sato S, Momiyama M, Mori R, Matsuyama R, Chishima T, Tanaka K, Ichikawa Y, Bouvet M, Endo I, Zhao M, Hoffman RM. 2014c. 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 5: 12346–12357.

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 2014d. 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, 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, Uehara F, Maawy A, Murakami T, Mii S, Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, Matsuyama R, Chishima T, Tanaka K, Ichikawa Y, Bouvet M, Endo I, Hoffman RM. 2015b. 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, Matsuyama R, Chishima T, Tanaka K, Ichikawa Y, Bouvet M, Endo I, Hoffman RM. 2015c. Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice. PLoS ONE 10:e0134324.

Hiroshima Y, Zhang Y, Zhang M, Maawy A, Mii S, Yamamoto M, Uehara F, Miwa S, Yano S, Murakami T, Momiyama M, Chishima T, Tanaka K, Ichikawa Y, Bouvet M, Endo I, Hoffman RM. 2015d. Establishment of a patient-derived orthotopic xenograph (PDOX) model of HER-2-positive cervical cancer expressing the clinical metastatic pattern. PLoS ONE 10(e):0117417.

Hiroshima Y, Zhang Y, Zhao M, Zhang N, Murakami T, Maawy A, Mii S, Uehara F, Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, Matsuyama R, Chishima T, Tanaka K, Ichikawa Y, Bouvet M, Endo I, Hoffman RM. 2015e. Tumor-targeting *Salmonella typhimurium* A1-R in combination with Trastuzumab eradicates HER-2-positive cervical cancer cells in patient-derived mouse models. PLoS ONE 10:e0120358.

Hoffman RM, Zhao M. 2014. Methods for the development of tumor-targeting bacteria. Expert Opin Drug Discov 9:741–750.

Hoffman RM. 2015. Patient-derived orthotopic xenografts: Better mimic of metastasis than subcutaneous xenografts. Nature Reviews Cancer 15: 451–452.

Hoffman RM, editor. 2016. Bacterial therapy of cancer: Methods and protocols. In: Walker JM, (series editor). Methods in molecular Biology 1409. Humana Press (Springer Science + Business Media, New York)

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 SD, Russell T, Dry SM, Li Y, Eilber FC, Hoffman RM. 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.

Kimura H, Zhang L, Zhao M, Hayashi K, Tsuchiya H, Tomita K, Bouvet M, Wessels J, Hoffman RM. 2010. Targeted therapy of spinal cord glioma with a genetically-modified *Salmonella typhimurium*. Cell Proliferation 43: 41–48.

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, Hoffman RM. 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 nude mouse model. Oncotarget 7:33046–33054.

Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, de la Cruz-Merino FL, Dutriaux C, Garbe C, Sovak MA, Chang I, Choong N, Hack SP, McArthur GA, Ribas A. 2014. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 371:1867–1876.

Liu F, Zhang L, Hoffman RM, Zhao M. 2010. Vessel destruction by tumor-targeting *Salmonella typhimurium* A1-R is enhanced by high tumor vascularity. Cell Cycle 9:4518–4524.

Matsumoto Y, Miwa S, Zhang Y, Hiroshima Y, Yano S, Uehara F, Yamamoto M, Toneri M, Bouvet M, Matsubara H, Hoffman RM, Zhao M. 2014. Efficacy of tumor-targeting *Salmonella typhimurium* A1-R on nude mouse models of metastatic and disseminated human ovarian cancer. J Cell Biochem 115:1996–2003.

Matsumoto Y, Miwa S, Zhang Y, Zhao M, Yano S, Uehara F, Yamamoto M, Hiroshima Y, Toneri M, Bouvet M, Matsubara H, Tsuchiya H, Hoffman RM. 2015. Intraperitoneal administration of tumor-targeting *Salmonella typhimurium* A1-R inhibits disseminated human ovarian cancer and extends survival in nude mice. Oncotarget 6:11369–11377.

McArthur GA, Chapman PB, Robert C, Larkin J, Haanen JB, Dummer R, Ribas A, Hogg D, Hamid O, Ascierto PA, Garbe C, Testori A, Maio M, Lorigan P, Lebbé C, Jouary T, Schadendorf D, O'Day SJ, Kirkwood JM, Eggermont AM, Dréno B, Sosman JA, Flaherty KT, Yin M, Caro I, Cheng S, Trunzer K, Hauschild A. 2014. Safety and efficacy of vemurafenib in BRAF (V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): Extended follow-up of a phase 3, randomised, open-label study. Lancet Oncol 15:323–332.

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.

Miwa S, Zhang Y, Baek K-E, Uehara F, Yano S, Yamamoto M, Hiroshima Y, Matsumoto Y, Kimura H, Hayashi K, Yamamoto N, Bouvet M, Tsuchiya H, Hoffman RM, Zhao M. 2014. Inhibition of spontaneous and experimental lung metastasis of soft-tissue sarcoma by tumor-targeting *Salmonella typhimurium* A1-R. Oncotarget 5:12849–12861.

Momiyama M, Zhao M, Kimura H, Tran B, Chishima T, Bouvet M, Endo I, Hoffman RM. 2012. Inhibition and eradication of human glioma with tumor-targeting *Salmonella typhimurium* in an orthotopic nude-mouse model. Cell Cycle 11:628–632.

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, Chishima T, Tanaka K, Bouvet M, Chawla S, Endo I, Hoffman RM. 2016a. 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, Singh AS, Kiyuna T, Dry SM, Li Y, James AW, Igarashi K, Kawaguchi K, DeLong JC, Zhang Y, Hiroshima Y, Russell T, Eckardt MA, Yanagawa J, Federman N, Matsuyama R, Chishima T, Tanaka K, Bouvet M, Endo I, Eilber FC, Hoffman RM. 2016b. 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.

Nagakura C, Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Bouvet M, Hoffman RM. 2009. Efficacy of a genetically-modified *Salmonella typhimurium* in an orthotopic human pancreatic cancer in nude mice. Anticancer Res 29:1873–1878.

Rabik CA, Dolan ME. 2007. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 33:9–23.

Slominski AT, Carlson JA. 2014. Melanoma resistance: A bright future for academicians and a challenge for patient advocates. Mayo Clin Proc 89:429–433.

Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A. 2012. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med 366:707–714.

Tang H, Wang Y, Chlewicki LK, Zhang Y, Guo J, Liang W, Wang J, Wang X, Fu YX. 2016. Facilitating T Cell Infiltration in Tumor Microenvironment Overcomes Resistance to PD-L1 Blockade. Cancer Cell 29:285–296.

Uchugonova A, Duong J, Zhang N, König K, Hoffman RM. 2011. The bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle. J Cell Biochem 112:2046–2050.

Uchugonova A, Zhao M, Zhang Y, Weinigel M, König K, Hoffman RM. 2012. Cancer-cell killing by engineered Salmonella imaged by multiphoton tomography in live mice. Anticancer Res 32:4331–4339.

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.

Yam C, Zhao M, Hayashi K, Ma H, Kishimoto H, McElroy M, Bouvet M, Hoffman RM. 2010. Monotherapy with a tumor-targeting mutant of S. typhimurium inhibits liver metastasis in a mouse model of pancreatic cancer. J Surg Res 164:248–255.

Yamamoto M, Zhao M, Hiroshima Y, Zhang Y, Shurell E, Eilber FC, 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, Zhang Y, Zhao M, Hiroshima Y, Miwa S, Uehara F, Kishimoto H, Tazawa H, Bouvet M, Fujiwara T, Hoffman RM. 2014. Tumor-targeting *Salmonella typhimurium* A1-R decoys quiescent cancer cells to cycle as visualized by FUCCI imaging and become sensitive to chemotherapy. Cell Cycle 13:3958–3963.

Zhao M, Yang M, Li XM, Jiang P, Baranov E, Li S, Xu M, Penman S, Hoffman RM. 2005. Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing *Salmonella typhimurium*. Proc Natl Acad Sci USA 102:755–760.

Zhao M, Yang M, Ma H, Li X, Tan X, Li S, Yang Z, Hoffman RM. 2006. Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. Cancer Res 66:7647–7652.

Zhao M, Geller J, Ma H, Yang M, Penman S, Hoffman RM. 2007. Monotherapy with a tumor-targeting mutant of *Salmonella typhimurium* cures orthotopic metastatic mouse models of human prostate cancer. Proc Natl Acad Sci USA 104:10170–10174.