IN VIVO *39*: 640-647 (2025)
doi: 10.21873/invivo.13869

Dynamic Fluorescence Imaging of Orally-administered Tumor-targeting *Salmonella typhimurium* A1-R Expressing Green Fluorescent Protein Trafficking Through the Gastrointestinal System to Target Fibrosarcomas in Nude Mice

SEI MORINAGA[1,2,3], MING ZHAO[1], KOHEI MIZUTA[1,2], BYUNG MO KANG[1,2], MICHAEL BOUVET[2], NORIO YAMAMOTO[3], KATSUHIRO HAYASHI[3], HIROAKI KIMURA[3], SHINJI MIWA[3], KENTARO IGARASHI[3], TAKASHI HIGUCHI[3], HIROYUKI TSUCHIYA[3], SATORU DEMURA[3] and ROBERT M. HOFFMAN[1,2]

[1]AntiCancer Inc., San Diego, CA, U.S.A.;
[2]Department of Surgery, University of California, San Diego, CA, U.S.A.;
[3]Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan

Abstract

Background/Aim: Salmonella typhimurium A1-R (A1-R) expresses green fluorescent protein (GFP) and has the ability to selectively target and inhibit all major cancer types in murine models without persistently infecting healthy tissue. A1-R is being developed for tumor targeting by oral administration. The aim of the present study was to demonstrate real-time imaging of orally-administered A1-R in a fibrosarcoma nude-mouse model and to visualize its trafficking through the gastrointestinal system to the tumor and normal organs.

Materials and Methods: A1-R-GFP (3.3×10^8 colony-forming units/ml) was administered orally to HT1080 human fibrosarcoma nude-mouse models which were fasted the day before administration. Fluorescence images of A1-R-GFP inside the gastrointestinal tract at 0, 2 and 4 hours after oral gavage were captured. The number of colonies of A1-R-GFP in tumors and liver were determined at 4 hours, and on days 1, 3 and 4 by growth from homogenized tumor and liver tissue on agar plates.

Results: The trafficking of A1-R-GFP through the murine gastrointestinal tract post-gavage was monitored in real-time *via* GFP fluorescence imaging. Bacteria, initially observed in the stomach, migrated to the small intestine and the colon and subsequently to the subcutaneously-implanted fibrosarcoma. A1-R-GFP proliferated in the tumors over time. In contrast, A1-R-GFP in the liver diminished over time.

Conclusion: The present study showed the pathway of orally administered A1-R-GFP in the gastrointestinal system and to the tumor and liver. A1-R selectively proliferated continuously in tumors and was cleared from the liver. These results are critical for future clinical trials of orally-administered A1-R-GFP.

Robert M. Hoffman, Ph.D., AntiCancer Inc, 7917 Ostrow St, Suite B, San Diego, CA, 92111, U.S.A. Tel: +1 6198852284, e-mail: all@anticancer.com

Received October 29, 2024 | Revised November 19, 2024 | Accepted December 2, 2024

Keywords: *Salmonella typhimurium* A1-R, green fluorescent protein (GFP), oral administration, gastrointestinal trafficking, dynamic fluorescence imaging, tumor-targeting, HT1080, fibrosarcoma, liver.

Introduction

Bacterial therapy of cancer has a history of more than 200 years. William B. Coley treated patients with sarcoma, starting 130 years ago, with live bacteria with much success (1-3), but was arbitrarily forced to stop in the 1930s. Since the end of the previous century, bacterial therapy of cancer has been developed pre-clinically including a few phase I clinical trials (4, 5).

We have engineered tumor-targeting *Salmonella typhimurium* A1-R (A1-R), tagged with green fluorescent protein (GFP), having auxotrophic mutations for leucine and arginine that prevent sustained infection in healthy tissues. A1-R has broad efficacy against all the main cancer types with minimal adverse effects in mouse models (6-45).

Jia *et al.* have shown the promise of oral administration of *Salmonella typhimurium* VNP20009 against cancers in mice (46, 47). The aim of the present study was to demonstrate real-time fluorescence imaging of orally-administered A1-R expressing GFP to visualize the trafficking of A1-R through the gastrointestinal system and subsequently to the liver and tumors in nude mice.

Materials and Methods

Mice. Nude mice (4-6 weeks, athymic nu/nu; AntiCancer Inc., San Diego, CA, USA) were utilized. All studies were performed in accordance with a protocol approved by the AntiCancer Institutional Animal Care and Use Committee, adhering to the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals (6-9). All experiments adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0.

Cells. HT1080 human fibrosarcoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY), supplemented with 10% fetal bovine serum and 1 IU/ml penicillin/streptomycin.

Preparation and administration of Salmonella typhimurium A1-R-GFP (AntiCancer Inc., San Diego, CA, USA)) were cultured overnight in Luria-Bertani (LB) medium (Fisher Sci., Hanover Park, IL) supplemented with ampicillin and subsequently diluted 1:10 in LB medium. Bacteria were collected during the late-log phase, rinsed with phosphate-buffered saline (PBS), and subsequently diluted in PBS for oral administration in nude-mouse models (6-9).

Establishment of HT1080 tumors in nude mice. Nude mice received a subcutaneous injection of HT1080 cells (1×10^6) in the right flank. Subcutaneous tumors were established by one month post-injection. The tumors were excised at one month and minced into 3-4 mm^3 fragments before being implanted into the right flank of additional nude mice. Subcutaneous tumors for experimentation were established 2 weeks later.

Real-time intravital imaging after oral administration of A1-R-GFP to the HT1080-fibrosarcoma nude-mouse model. Mice with HT1080 tumors received a single oral dose of 3.3×10^8 colony-forming units (CFU) of A1-R-GFP in 100 μl PBS. The mice were fasted 1 day prior to oral administration. Images were captured inside the peritoneum of the mice at 0, 2 and 4 hours after A1-R-GFP administration, using a BioSpectrum Advanced 900 (Analytik Jena US LLC, Upland, CA, USA).

Quantification of Salmonella typhimurium A1-R in tumors and liver following oral administration. Mice with HT1080 tumors (n=3 per timepoint) received 3.3×10^8 CFU A1-R-GFP *via* oral administration. The subcutaneous tumor and

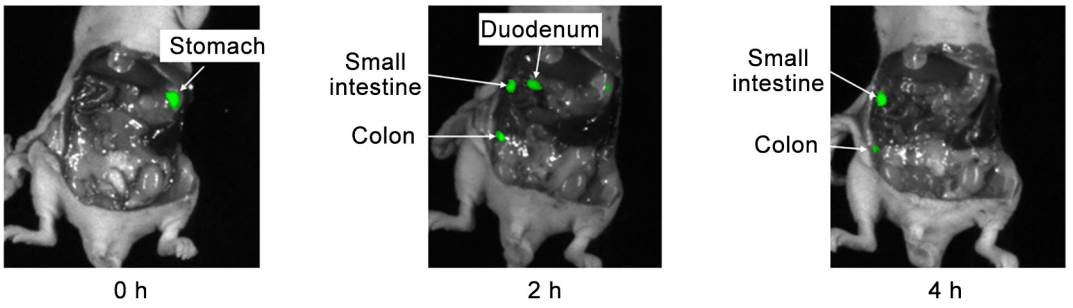

Figure 1. *Real-time intravital fluorescence imaging of Salmonella typhimurium A1-R-GFP. Imaging was performed 0, 2 and 4 hours after oral administration of A1-R. GFP: Green fluorescent protein. Please see Materials and Methods for details.*

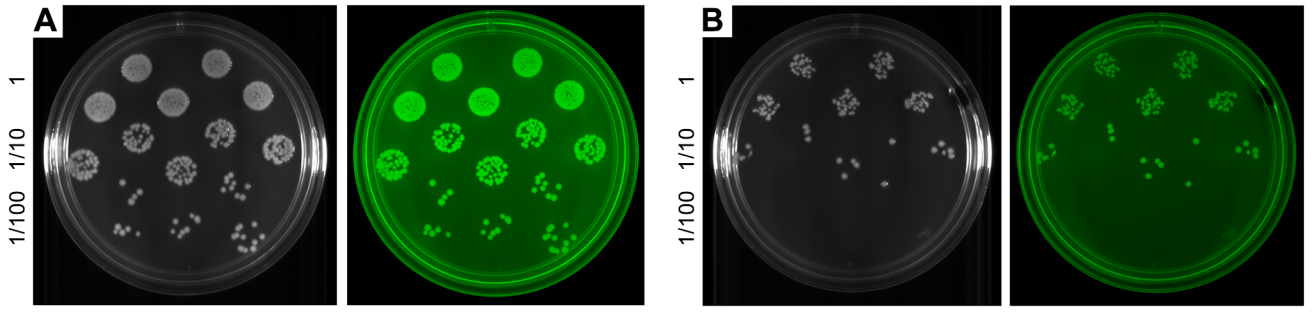

Figure 2. *Representative images of GFP-labeled Salmonella typhimurium A1-R cultured (A1-R-GFP) on LB agar from the tumors and the livers of mice on day 1 after oral administration of A1-R-GFP. (A) Bright-field (left) and fluorescence (right) images of A1-R-GFP colonies grown from tumors on LB agar. (B) Bright-field (left) and fluorescence (right) images of A1-R-GFP colonies grown on LB agar from livers. The numbers at the side indicate the degree of dilution. GFP: Green fluorescent protein. Please see Materials and Methods for details.*

liver were excised after 4 hours, 24 hours, 3 days and 4 days after A1-R-GFP administration. The tumor and liver were homogenized, and their supernatants were cultured on LB agar containing ampicillin. The quantity of infectious bacteria per gram of tumor or organ was determined.

Results

Real-time imaging of orally administered A1-R-GFP in a nude-mouse model of fibrosarcoma. The trafficking of A1-R-GFP through the murine gastrointestinal tract post-gavage was monitored in real time *via* intravital imaging. Bacteria were initially observed in the stomach, migrated to the duodenum, small intestine and colon (Figure 1).

Quantification of A1-R-GFP in the tumor and liver. A1-R-GFP derived from excised tumor tissue and cultured on LB agar increased over time following oral administration of A1-R-GFP. In contrast, the quantity of colonies that were cultured from the excised liver diminished over time following the oral administration of A1-R-GFP (Figure 2 and Figure 3).

Discussion

For at least 200 years, it has been documented that bacterial infections can induce regression of cancer in patients. During the late 19th and early 20th centuries, Dr. William B. Coley administered bacteria to patients with cancer with much success (1-3). Coley was arbitrarily forced to cease bacterial therapy of cancer in the 1930s. Since the end of the last century, bacterial therapy has been researched

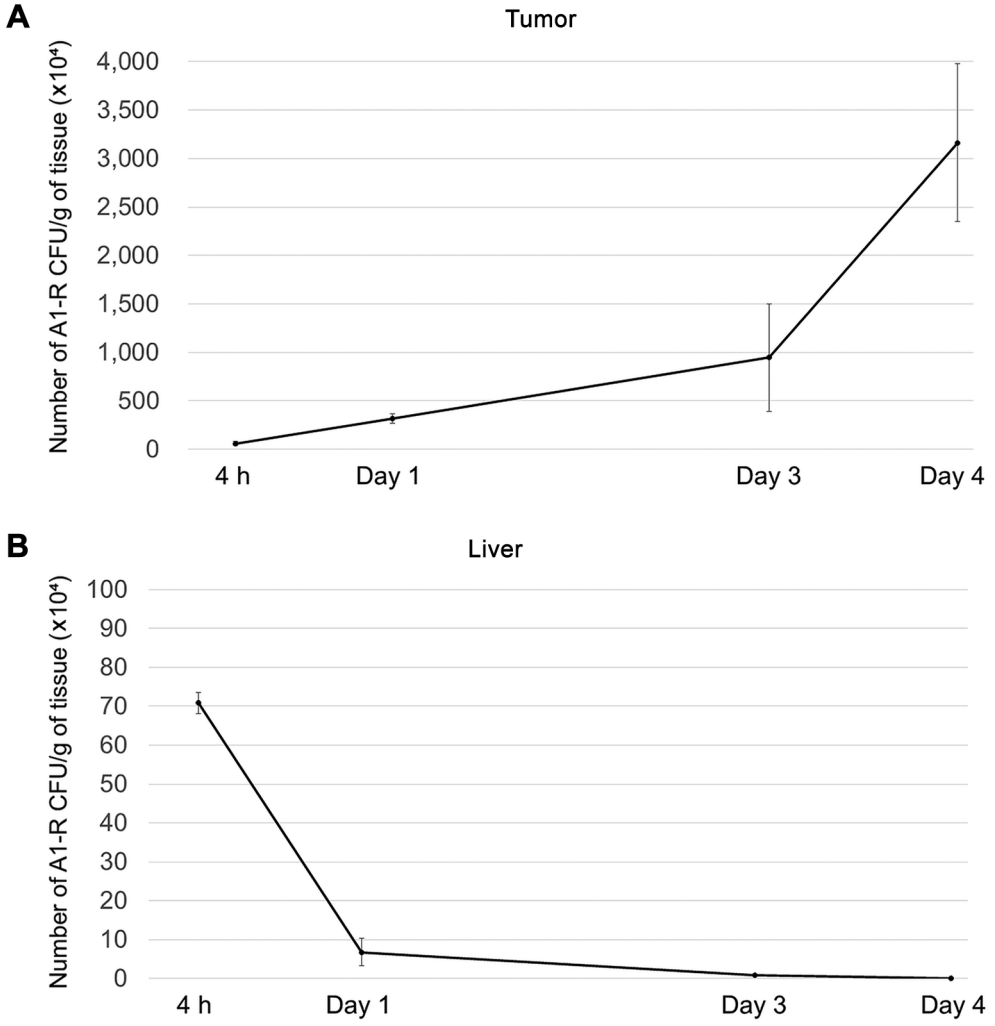

Figure 3. *Number of Salmonella typhimurium A1-R-GFP colony-forming units (CFU) in tumors and liver after oral administration of A1-R-GFP (mean±standard deviation). A: The number of A1-R-GFP colonies derived from the tumor, increased over time when cultured on LB agar. B: In contrast, the number of A1-R colonies derived from the liver decreased over time when cultured on LB agar. Please see Materials and Methods for details.*

pre-clinically with *Salmonella typhimurium*, with a phase I clinical trial of another strain of *Salmonella typhimurium* VNP20009 (48, 49). In our laboratory, A1-R inhibited and eradicated metastatic cancer when administered *via* multiple routes in murine models (6-45).

Our goal is to develop A1-R as an oral probiotic against cancer. In the present study, the transition of A1-R-GFP through the murine gastrointestinal tract post-gavage was monitored by intravital real-time fluorescence imaging (50). Bacteria were initially observed in the stomach, migrated to the small intestine and to the colon, shown by intravital direct imaging. A1-R-GFP can target and grow within tumors and promote T-cell infiltration into the tumor and this can promote cancer immunotherapy (45).

A1-R is a specific autotrophic strain of *Salmonella typhimurium* developed by our laboratory which requires leucine and arginine for proliferation. A1-R can proliferate in the presence or absence of oxygen and targets all tumor types tested and inhibits their growth (6-45). The specific nutritional requirements of A1-R are satisfied in the

nutrient-dense environment of tumors, whereas its growth is considerably restricted in normal tissue due to its auxotrophy (6-8). The present results showed while the number of A1-R colonies increased over time in the tumor, they disappeared in the liver within 4 days.

The present findings indicate the potential of orally administered A1-R as a probiotic for the treatment of cancer. A strain of Salmonella typhimurium expressing human IL-2 has been tested in humans via oral administration in a phase I clinical trial without toxicity or adverse events (51).

Conflicts of Interest

The Authors declare no competing interests.

Authors' Contributions

SM and RMH designed the study. SM performed experiments. SM was a major contributor to writing the manuscript and RMH revised the manuscript. MZ, KM, BMK, MS, MB, NY, KH, HK, SM, KI, TH, HT and SD critically read and approved the final manuscript.

Acknowledgements

This paper is dedicated to the memory of A.R. Moosa, MD, Sun Lee, MD, Professor Gordon H. Sato, Professor Li Jiaxi, Masaki Kitajima, MD, Shigeo Yagi, Ph.D., Jack Geller, MD, Joseph R Bertino, MD, J.A.R. Mead, Ph.D., Eugene P. Frenkel, MD, Professor Lev Bergelson, Professor Sheldon Penman, Professor John R. Raper, John Mendelsohn, MD and Joseph Leighton, MD.

References

1 Coley WB: The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the Streptococcus erysipelas and the Bacillus prodigiosus). Proc R Soc Med 3: 1-48, 1910.

2 Hoption Cann SA, van Netten JP, van Netten C: Dr William Coley and tumour regression: A place in history or in the future. Postgrad Med J 79: 672-680, 2003.

3 Richardson MA, Ramirez T, Russell NC, Moye LA: Coley toxins immunotherapy: A retrospective review. Altern Ther Health Med 5: 42-47, 1999.

4 Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, Adler K, Dick-Necula D, Raskin S, Bloch N, Rotin D, Anafi L, Avivi C, Melnichenko J, Steinberg-Silman Y, Mamtani R, Harati H, Asher N, Shapira-Frommer R, Brosh-Nissimov T, Eshet Y, Ben-Simon S, Ziv O, Khan MAW, Amit M, Ajami NJ, Barshack I, Schachter J, Wargo JA, Koren O, Markel G, Boursi B: Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 371(6529): 602-609, 2021. DOI: 10.1126/science.abb5920

5 Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, Deblasio RN, Menna C, Ding Q, Pagliano O, Zidi B, Zhang S, Badger JH, Vetizou M, Cole AM, Fernandes MR, Prescott S, Costa RGF, Balaji AK, Morgun A, Vujkovic-Cvijin I, Wang H, Borhani AA, Schwartz MB, Dubner HM, Ernst SJ, Rose A, Najjar YG, Belkaid Y, Kirkwood JM, Trinchieri G, Zarour HM: Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 371(6529): 595-602, 2021. DOI: 10.1126/science.abf3363

6 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 U.S.A. 102(3): 755-760, 2005. DOI: 10.1073/pnas.0408422102

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 66(15): 7647-7652, 2006. DOI: 10.1158/0008-5472.CAN-06-0716

8 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 U.S.A. 104(24): 10170-10174, 2007. DOI: 10.1073/pnas.0703867104

9 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 32(7): 2501-8, 2012.

10 Zhang Y, Miwa S, Zhang N, Hoffman RM, Zhao M: Tumor-targeting Salmonella typhimurium A1-R arrests growth of breast-cancer brain metastasis. Oncotarget 6(5): 2615-2622, 2015. DOI: 10.18632/oncotarget.2811

11 Uchugonova A, Zhao M, Zhang Y, Weinigel M, König K and Hoffman RM: Cancer-cell killing by engineered Salmonella imaged by multiphoton tomography in live mice. Anticancer Res 32(10): 4331-4337, 2012.

12 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 9(22): 4518-4524, 2010. DOI: 10.4161/cc.9.22.13744

13 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 29(6): 1873-1878, 2009.

14 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 164(2): 248-255, 2010. DOI: 10.1016/j.jss.2009.02.023

15 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: Comparison of efficacy of Salmonella typhimurium A1-R and chemotherapy on stem-like and non-stem human pancreatic cancer cells. Cell Cycle 12(17): 2774-2780, 2013. DOI: 10.4161/cc.25872

16 Matsumoto Y, Miwa S, Zhang Y, Hiroshima Y, Yano S, Uehara F, Yamamoto M, Toneri M, Bouvet M, Matsubara H, Hoffman RM, Zhao M: Efficacy of tumor-targeting Salmonella typhimurium A1-R on nude mouse models of metastatic and disseminated human ovarian cancer. J Cell Biochem 115(11): 1996-2003, 2014. DOI: 10.1002/jcb.24871

17 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: Intraperitoneal administration of tumor-targeting Salmonella typhimurium A1-R inhibits disseminated human ovarian cancer and extends survival in nude mice. Oncotarget 6(13): 11369-11377, 2015. DOI: 10.18632/oncotarget.3607

18 Yano S, Zhang Y, Zhao M, Hiroshima Y, Miwa S, Uehara F, Kishimoto H, Tazawa H, Bouvet M, Fujiwara T, Hoffman RM: 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(24): 3958-3963, 2014. DOI: 10.4161/15384101.2014.964115

19 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: 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(6): e0120358, 2015. DOI: 10.1371/journal.pone.0120358

20 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: Tumor-targeting Salmonella typhimurium A1-R in combination with doxorubicin eradicate soft tissue sarcoma in a patient-derived orthotopic xenograft (PDOX) model. Oncotarget 7(11): 12783-12790, 2016. DOI: 10.18632/oncotarget.7226

21 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: Tumor-targeting Salmonella typhimurium A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice. PLoS One 10(8): e0134324, 2015. DOI: 10.1371/journal.pone.0134324

22 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: High efficacy of tumor-targeting Salmonella typhimurium A1-R on a doxorubicin- and dactolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model. Oncotarget 7(22): 33046-33054, 2016. DOI: 10.18632/oncotarget.8848

23 Yamamoto M, Zhao M, Hiroshima Y, Zhang Y, Shurell E, Eilber FC, Bouvet M, Noda M, Hoffman RM: Efficacy of tumor-targeting Salmonella A1-R on a melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. PLoS One 11(8): e0160882, 2016. DOI: 10.1371/journal.pone.0160882

24 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 11(3): 628-632, 2012. DOI: 10.4161/cc.11.3.19116

25 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: Efficacy of Salmonella typhimurium A1-R *versus* chemotherapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX). J Cell Biochem 115(7): 1254-1261, 2014. DOI: 10.1002/jcb.24769

26 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 typhymurium. Cell Cycle 8(6): 870-875, 2009. DOI: 10.4161/cc.8.6.7891

27 Igarashi K, Kawaguchi K, Murakami T, Kiyuna T, Miyake K, Nelson SD, Dry SM, Li Y, Yanagawa J, Russell TA, Singh AS, Yamamoto N, Hayashi K, Kimura H, Miwa S, Tsuchiya H, Eilber FC, Hoffman RM: Intra-arterial administration of tumor-targeting Salmonella typhimurium A1-R regresses a cisplatin-resistant relapsed osteosarcoma in a patient-derived orthotopic xenograft (PDOX) mouse model. Cell Cycle 16(12): 1164-1170, 2017. DOI: 10.1080/15384101.2017.1317417

28 Kawaguchi K, Igarashi K, Murakami T, Chmielowski B, Kiyuna T, Zhao M, Zhang Y, Singh A, Unno M, Nelson SD, Russell TA, Dry SM, Li Y, Eilber FC, Hoffman RM: 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 7(52): 85929-85936, 2016. DOI: 10.18632/oncotarget.13231

29 Igarashi K, Kawaguchi K, Murakami T, Miyake K, Kiyuna T, Miyake M, Hiroshima Y, Higuchi T, Oshiro H, Nelson SD, Dry SM, Li Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Singh SR, Tsuchiya H, Hoffman RM: Patient-derived orthotopic xenograft

models of sarcoma. Cancer Lett 469: 332-339, 2020. DOI: 10.1016/j.canlet.2019.10.028
30 Igarashi K, Kawaguchi K, Zhao M, Kiyuna T, Miyake K, Miyake M, Nelson SD, Dry SM, Li Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Higuchi T, Singh SR, Tsuchiya H, Hoffman RM: Exquisite tumor targeting by Salmonella A1-R in combination with caffeine and valproic acid regresses an adult pleomorphic rhabdomyosarcoma patient-derived orthotopic xenograft mouse model. Transl Oncol 13(2): 393-400, 2020. DOI: 10.1016/j.tranon.2019.10.005
31 Yano S, Takehara K, Zhao M, Tan Y, Han Q, Li S, Bouvet M, Fujiwara T, Hoffman RM: Tumor-specific cell-cycle decoy by Salmonella typhimurium A1-R combined with tumor-selective cell-cycle trap by methioninase overcome tumor intrinsic chemoresistance as visualized by FUCCI imaging. Cell Cycle 15(13): 1715-1723, 2016. DOI: 10.1080/15384101.2016.1181240
32 Miyake K, Murata T, Murakami T, Zhao M, Kiyuna T, Kawaguchi K, Igarashi K, Miyake M, Lwin TM, Hozumi C, Komatsu S, Kikuchi T, Bouvet M, Shimoya K, Singh SR, Endo I, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R overcomes nab-paclitaxel resistance in a cervical cancer PDOX mouse model. Arch Gynecol Obstet 299(6): 1683-1690, 2019. DOI: 10.1007/s00404-019-05147-3
33 Hamada K, Aoki Y, Yamamoto J, Hozumi C, Zhao M, Murata T, Sugisawa N, Bouvet M, Tsunoda T, Hoffman RM: Salmonella typhimurium A1-R exquisitely targets and arrests a matrix-producing triple-negative breast carcinoma in a PDOX model. In Vivo 35(6): 3067-3071, 2021. DOI: 10.21873/invivo.12602
34 Murakami T, Hiroshima Y, Miyake K, Kiyuna T, Endo I, Zhao M, Hoffman RM: Efficacy of tumor-targeting Salmonella typhimurium A1-R against malignancies in patient-derived orthotopic xenograft (PDOX) murine models. Cells 8(6): 599, 2019. DOI: 10.3390/cells8060599
35 Igarashi K, Kawaguchi K, Kiyuna T, Miyake K, Miyake M, Li S, Han Q, Tan Y, Zhao M, Li Y, Nelson SD, Dry SM, Singh AS, Elliott IA, Russell TA, Eckardt MA, Yamamoto N, Hayashi K, Kimura H, Miwa S, Tsuchiya H, Eilber FC, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R combined with recombinant methioninase and cisplatinum eradicates an osteosarcoma cisplatinum-resistant lung metastasis in a patient-derived orthotopic xenograft (PDOX) mouse model: decoy, trap and kill chemotherapy moves toward the clinic. Cell Cycle 17(6): 801-809, 2018. DOI: 10.1080/15384101.2018.1431596
36 Miyake K, Kawaguchi K, Miyake M, Zhao M, Kiyuna T, Igarashi K, Zhang Z, Murakami T, Li Y, Nelson SD, Bouvet M, Elliott I, Russell TA, Singh AS, Hiroshima Y, Momiyama M, Matsuyama R, Chishima T, Singh SR, Endo I, Eilber FC, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R suppressed an imatinib-resistant gastrointestinal stromal tumor with c-kit exon 11 and 17 mutations. Heliyon 4(6): e00643, 2018. DOI: 10.1016/j.heliyon.2018.e00643
37 Miyake K, Kiyuna T, Miyake M, Zhao M, Wangsiricharoen S, Kawaguchi K, Zhang Z, Higuchi T, Razmjooei S, Li Y, Nelson SD, Russell T, Singh A, Murakami T, Hiroshima Y, Momiyama M, Matsuyama R, Chishima T, Singh SR, Chawla SP, Eilber FC, Endo I, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R overcomes partial carboplatinum-resistance of a cancer of unknown primary (CUP). Tissue Cell 54: 144-149, 2018. DOI: 10.1016/j.tice.2018.09.001
38 Kiyuna T, Tome Y, Uehara F, Murakami T, Zhang Y, Zhao M, Kanaya F, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R inhibits osteosarcoma angiogenesis in the *in vivo* Gelfoam® assay visualized by color-coded imaging. Anticancer Res 38(1): 159-164, 2018. DOI: 10.21873/anticanres.12203
39 Kiyuna T, Tome Y, Murakami T, Zhao M, Miyake K, Igarashi K, Kawaguchi K, Miyake M, Oshiro H, Higuchi T, Li Y, Dry SM, Nelson SD, Russell TA, Eckardt MA, Singh AS, Kanaya F, Eilber FC, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R arrests a doxorubicin-resistant PDGFRA-amplified patient-derived orthotopic xenograft mouse model of pleomorphic liposarcoma. J Cell Biochem 119(9): 7827-7833, 2018. DOI: 10.1002/jcb.27183
40 Kawaguchi K, Miyake K, Zhao M, Kiyuna T, Igarashi K, Miyake M, Higuchi T, Oshiro H, Bouvet M, Unno M, Hoffman RM: Tumor targeting Salmonella typhimurium A1-R in combination with gemcitabine (GEM) regresses partially GEM-resistant pancreatic cancer patient-derived orthotopic xenograft (PDOX) nude mouse models. Cell Cycle 17(16): 2019-2026, 2018. DOI: 10.1080/15384101.2018.1480223
41 Kiyuna T, Tome Y, Murakami T, Miyake K, Igarashi K, Kawaguchi K, Oshiro H, Higuchi T, Miyake M, Sugisawa N, Zhang Z, Razmjooei S, Wangsiricharoen S, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Eckardt MA, Singh AS, Chawla S, Kanaya F, Eilber FC, Singh SR, Zhao M, Hoffman RM: A combination of irinotecan/cisplatinum and irinotecan/temozolomide or tumor-targeting Salmonella typhimurium A1-R arrest doxorubicin- and temozolomide-resistant myxofibrosarcoma in a PDOX mouse model. Biochem Biophys Res Commun 505(3): 733-739, 2018. DOI: 10.1016/j.bbrc.2018.09.106
42 Miyake K, Kiyuna T, Miyake M, Kawaguchi K, Yoon SN, Zhang Z, Igarashi K, Razmjooei S, Wangsiricharoen S, Murakami T, Li Y, Nelson SD, Russell TA, Singh AS, Hiroshima Y, Momiyama M, Matsuyama R, Chishima T, Singh SR, Endo I, Eilber FC, Hoffman RM: Patient-derived orthotopic xenograft models for cancer of unknown primary precisely distinguish chemotherapy, and tumor-targeting S. typhimurium A1-R is superior to first-line chemotherapy. Signal Transduct Target Ther 3: 12, 2018. DOI: 10.1038/s41392-018-0016-7
43 Miyake K, Kiyuna T, Li S, Han Q, Tan Y, Zhao M, Oshiro H, Kawaguchi K, Higuchi T, Zhang Z, Razmjooei S, Barangi M, Wangsiricharoen S, Murakami T, Singh AS, Li Y, Nelson SD, Eilber FC, Bouvet M, Hiroshima Y, Chishima T, Matsuyama R, Singh SR, Endo I, Hoffman RM: Combining tumor-selective

bacterial therapy with Salmonella typhimurium A1-R and cancer metabolism targeting with oral recombinant methioninase regressed an Ewing's sarcoma in a patient-derived orthotopic xenograft model. Chemotherapy 63(5): 278-283, 2018. DOI: 10.1159/000495574

44 Kawaguchi K, Higuchi T, Li S, Han Q, Tan Y, Igarashi K, Zhao M, Miyake K, Kiyuna T, Miyake M, Ohshiro H, Sugisawa N, Zhang Z, Razmjooei S, Wangsiricharoen S, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Eckardt MA, Singh AS, Singh SR, Eilber FC, Unno M, Hoffman RM: Combination therapy of tumor-targeting Salmonella typhimurium A1-R and oral recombinant methioninase regresses a BRAF-V600E-negative melanoma. Biochem Biophys Res Commun 503(4): 3086-3092, 2018. DOI: 10.1016/j.bbrc.2018.08.097

45 Murakami T, Hiroshima Y, Zhang Y, Zhao M, Kiyuna T, Hwang HK, Miyake K, Homma Y, Mori R, Matsuyama R, Chishima T, Ichikawa Y, Tanaka K, Bouvet M, Endo I, Hoffman RM: Tumor-targeting Salmonella typhimurium A1-R promotes tumoricidal CD8+ T-cell tumor infiltration and arrests growth and metastasis in a syngeneic pancreatic-cancer orthotopic mouse model. J Cell Biochem 119: 634-639, 2018. DOI: 10.1002/jcb.26224

46 Jia LJ, Wei DP, Sun QM, Huang Y, Wu Q, Hua ZC: Oral delivery of tumor-targeting Salmonella for cancer therapy in murine tumor models. Cancer Sci 98(7): 1107-1112, 2007. DOI: 10.1111/j.1349-7006.2007.00503.x

47 Chen G, Wei DP, Jia LJ, Tang B, Shu L, Zhang K, Xu Y, Gao J, Huang XF, Jiang WH, Hu QG, Huang Y, Wu Q, Sun ZH, Zhang JF, Hua ZC: Oral delivery of tumor-targeting Salmonella exhibits promising therapeutic efficacy and low toxicity. Cancer Sci 100(12): 2437-2443, 2009. DOI: 10.1111/j.1349-7006.2009.01337.x

48 Zhang Y, Cao W, Toneri M, Zhang N, Kiyuna T, Murakami T, Nelson SD, Dry SM, Li Y, Li S, Wang X, Ma H, Singh AS, Eilber FC, Hoffman RM, Zhao M: Toxicology and efficacy of tumor-targeting Salmonella typhimurium A1-R compared to VNP 20009 in a syngeneic mouse tumor model in immuno-competent mice. Oncotarget 8(33): 54616-54628, 2017. DOI: 10.18632/oncotarget.17605

49 Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, Sherry RM, Topalian SL, Yang JC, Stock F, Freezer LJ, Morton KE, Seipp C, Haworth L, Mavroukakis S, White D, MacDonald S, Mao J, Sznol M, Rosenberg SA: Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma. J Clin Oncol 20(1): 142-152, 2002. DOI: 10.1200/JCO.2002.20.1.142

50 Zhao M, Yang M, Baranov E, Wang X, Penman S, Moossa AR, Hoffman RM: Spatial-temporal imaging of bacterial infection and antibiotic response in intact animals. Proc Natl Acad Sci USA 98: 9814-9818, 2001. DOI: 10.1073/pnas.161275798

51 Gniadek TJ, Augustin L, Schottel J, Leonard A, Saltzman D, Greeno E, Batist G: A phase I, dose escalation, single dose trial of oral attenuated Salmonella typhimurium containing human IL-2 in patients with metastatic gastrointestinal cancers. J Immunother 43(7): 217-221, 2020. DOI: 10.1097/CJI.0000000000000325