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Combination therapy of tumor-targeting *Salmonella typhimurium* A1-R and oral recombinant methioninase regresses a BRAF-V600E-negative melanoma

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ABSTRACT

Melanoma is a recalcitrant cancer. To improve and individualize treatment for this disease, we previously developed a patient-derived orthotopic xenograft (PDOX) model for melanoma. We previously reported the individual efficacy of tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) and recombinant methioninase (rMETase) for melanoma in the PDOX models of this disease. In the present study, we evaluated the efficacy of the combination of *S. typhimurium* A1-R with orally-administered rMETase (o-rMETase) for BRAF-V600E-negative melanoma in a PDOX model. Three weeks after implantation, 60 PDOX mouse models were randomized into six groups of 10 mice each: untreated control, temozolomide (TEM); o-rMETase; *S. typhimurium* A1-R; TEM + rMETase, *S. typhimurium* A1-R + rMETase. All treatments inhibited tumor growth compared to untreated control (TEM: $p < 0.0001$, rMETase: $p < 0.0001$, *S. typhimurium* A1-R: $p < 0.0001$, TEM + rMETase: $p < 0.0001$, *S. typhimurium* A1-R + rMETase: $p < 0.0001$). The most effective was the combination of *S. typhimurium* A1-R + o-rMETase which regressed this melanoma PDOX, thereby indicating a new paradigm for treatment of metastatic melanoma.

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1. Introduction

Melanoma is a recalcitrant and one of the most common malignancies in the United States. It is accountable for most of skin-cancer-related deaths. The American Cancer Society has estimated that approximately 91,270 new cases of melanomas will be diagnosed and about 9320 people are expected to die because of this disease in the coming year [1]. Melanoma has been categorized into 4 genomic subtypes such as mutant v-raf murine sarcoma viral oncogene homolog B (BRAF), mutant neuroblastoma RAS viral oncogene homolog (NRAS), mutant neurofibromatosis type 1 (NF1), and triple-wild-type (3xWT) [2]. BRAF-targeting drugs have been shown to inhibit the activated BRAF-mutated kinase [3]. In addition, the combination of targeted inhibitors has been reported

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to significantly improve the overall survival in patients with BRAF-mutant melanoma [4]. Melanoma is recalcitrant to most available chemotherapies, therefore, targeted therapy is urgently needed. To improve and individualize treatment for this disease, we developed a patient-derived orthotopic xenograft (PDOX) model for melanoma [5–14].

We previously reported the individual efficacy of tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) and recombinant methioninase (rMETase) for melanoma in the PDOX models of this disease [5–14]. It is already known that cancers of all types require methionine (MET) at higher levels than normal cells to proliferate and survive [15–17]. We previously showed that intra-tumor methionine levels highly correlates with tumor size, further demonstrating the dependence of tumor growth on MET [14].

rMETase, a *Pseudomonas putida* enzyme cloned in *E. coli*, has been developed to target the methionine dependence of cancer. rMETase has been previously shown to be active for PDOX models of major cancers [13,14,18–26]. Oral administration of o-rMETase was shown to be more effective than intra-peritoneal injection in a melanoma PDOX model [19].

Tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) was developed by our laboratory [27–29]. *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 been shown to be effective in PDOX models of major cancers [7–9,30,31]. We previously reported that temozolomide (TEM) combined with *S. typhimurium* A1-R could inhibit tumor growth in PDOX model of the BRAF-V600E mutant melanoma [7].

In the present study, we evaluated the efficacy of the combination of *S. typhimurium* A1-R with orally-administered rMETase (o-rMETase) on a PDOX model for BRAF-V600E-negative melanoma in comparison with standard first-line therapy for this disease.

2. Materials and methods

2.1. Mice

In this study, athymic *nu/nu* male nude mice (AntiCancer, Inc., San Diego, CA), 4–6 weeks old, were used. Experimental procedures and data collection were done as per as our previous publications [30,31]. Mice accommodation, feeding, surgical processes and imaging were conducted as described [30,31]. To minimize any suffering of the animals, anesthesia and analgesics were used for all surgical experiments. The mouse investigations presented here were done using an AntiCancer, Inc. Institutional Animal Care and Use Committee (IACUC) protocol specifically approved for this study as previously described [32] and as per as principles and procedures provided in the National Institute of Health (NIH) Guide for the Care and Use of Animals under Assurance Number A3873-1 [32].

2.2. Patient-derived tumor

A patient was previously diagnosed with a BRAF-600E-negative melanoma of the abdominal 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 [7].

2.3. Establishment of PDOX models of melanoma by surgical orthotopic implantation (SOI)

All experimental protocols and data were collected as described [29,30]. Tumor collection from the melanoma patient and transportation to AntiCancer, Inc. have been described [7]. Procedures for making small fragments from tumor samples for subcutaneous transplantation in nude mice have been described [7]. After three weeks, the subcutaneously-implanted tumors grew to more than 10 mm. After subcutaneously-grown tumors reached 10 mm in diameter, they were harvested and cut into small fragments as described [7] and implanted orthotopically to establish the PDOX model as described [7]. The procedure to close wounds has been described [7].

2.4. Recombinant methioninase (rMETase) production

Recombinant L-methionine α-deamino-γ-mercaptomethane lyase (methioninase, METase) [EC 4.4.1.11] from *Pseudomonas putida* has been previously cloned and produced in *Escherichia coli* (AntiCancer, Inc.) and purified as previously described [33].

2.5. 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, USA) and then diluted 1:10 in LB medium. Bacteria were harvested at late-log phase, washed with PBS, and then diluted in PBS [27–29].

2.6. Treatment study design in the PDOX model of melanoma

PDOX mouse models were randomized into six groups of 10 mice each (Fig. 1A): untreated control (n = 10); TEM (25 mg/kg, oral (p.o.), 14 consecutive days, n = 10); rMETase (50 units, [p.o.], twice a day for 14 consecutive days, n = 10); *S. typhimurium* A1-R (5×10^7 CFU/100 μl, intravenous (i.v.), once a week for 2 weeks, n = 10), TEM + rMETase (TEM: 25 mg/kg, p.o., rMETase: 50 units, p.o., twice a day for 14 consecutive days, n = 10), *S. typhimurium* A1-R + rMETase (*S. typhimurium* A1-R: 5×10^7 CFU/100 μl, i.v., rMETase: 50 units, p.o., twice a day for 14 consecutive days, n = 10). Tumor length and width were measured twice a week. Tumor-volume was calculated as described in our previous publication [7]. Data are presented as mean ± SD. The tumor volume ratio is defined at the tumor volume at each point relative to pre-treatment tumor volume.

2.7. Imaging the melanoma PDOX

Imaging of the macroscopic tumor was performed with the OV100 Small Animal Imaging System (Olympus, Tokyo, Japan).

2.8. Bacterial cultured from tumor tissue and confocal microscopy

To verify *S. typhimurium* A1-R, a green fluorescent protein (GFP) in the *S. typhimurium* A1-R-GFP-treated tumors, the melanoma PDOX tumors treated with *S. typhimurium* A1-R were resected on day 22 to culture bacteria expressing GFP. The tumor specimens were homogenized and suspended in 1 ml PBS. The suspension was diluted 10 times each up to 1:10000, then cultured in LB agar medium for 12 h. The FV1000 confocal microscope (Olympus, Tokyo, Japan) was used for high-resolution imaging. Fluorescence images were obtained using the 20 × /0.5 UPlan FLN and 40 × /1.3 oil Olympus UPLAN FLN objectives [6–9,11–14,34].

2.9. Tumor histology

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (5 μm)

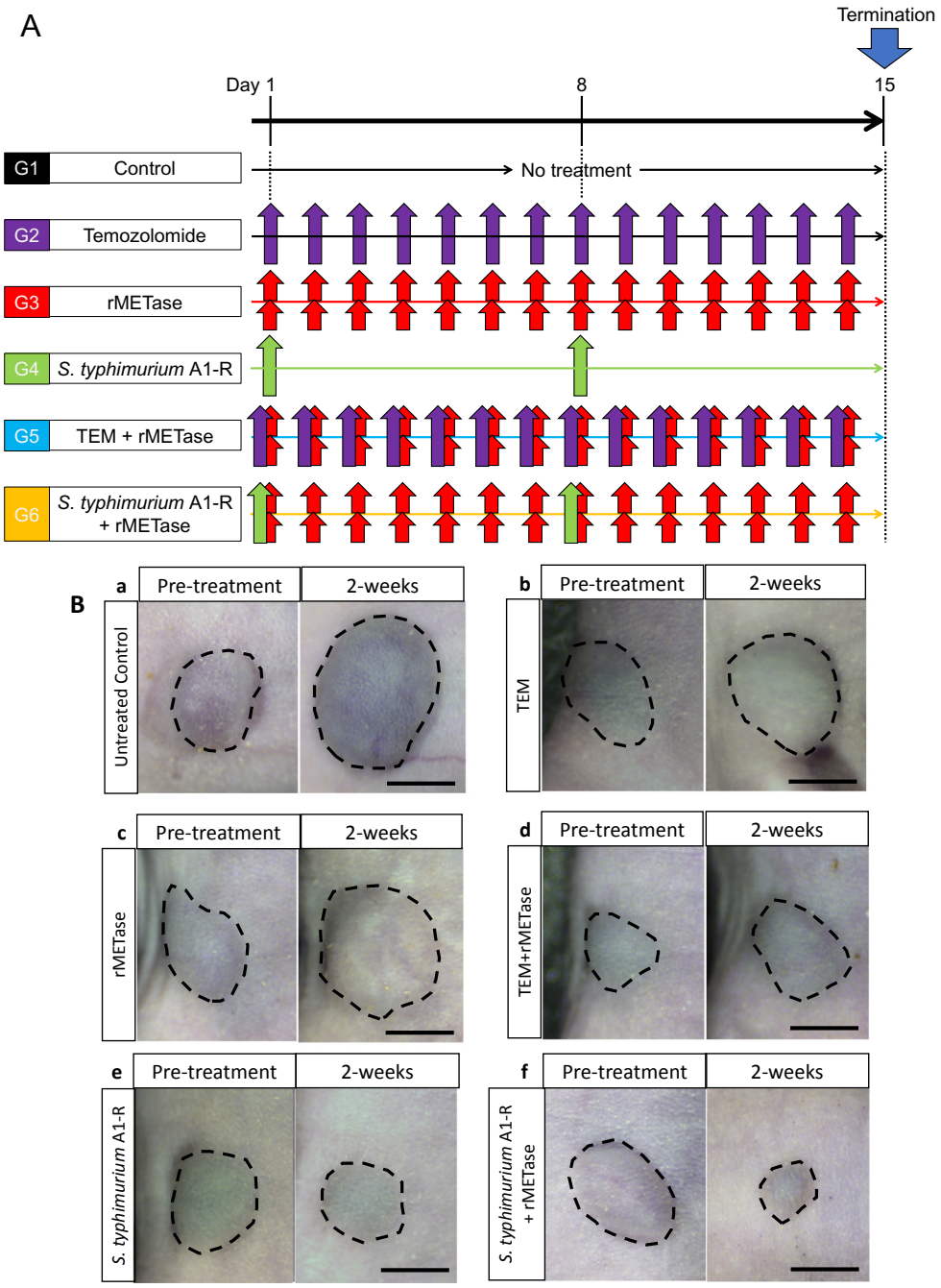

Fig. 1. (A) Treatment schema, G1: untreated control (n = 10); G2: TEM (n = 10); rMETase (n = 10); *S. typhimurium* A1-R (n = 10), TEM + rMETase (n = 10), *S. typhimurium* A1-R + rMETase (n = 10). (B) Imaging the treated and untreated resected melanoma PDOX tumors. (a) Tumor in untreated control. (b) Temozolomide (TEM) treated. (c) Oral recombinant methioninase (o-rMETase) treated. (d) Tumor treated with the combination of TEM and o-rMETase. (e) *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R treated). (f) Tumor treated with combination of *S. typhimurium* A1-R and o-rMETase. Scale bar: 5 mm.

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) [6–9,11–14].

2.10. Statistical analysis

JMP version 14.0 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 bars show SD. A probability value of $P \leq 0.05$ is considered statistically significant.

3. Results

3.1. Efficacy of S. typhimurium A1-R and rMETase

We compared the efficacy of known combinations [7] and *S. typhimurium A1-R* in combination with oral rMETase (o-rMETase). All treatments inhibited the melanoma PDOX tumor growth

compared to untreated control: TEM, $p < 0.0001$; o-rMETase, $p < 0.0001$; *S. typhimurium* A1-R, $p < 0.0001$; TEM + o-rMETase, $p < 0.0001$; *S. typhimurium* A1-R + o-rMETase, $p < 0.0001$, on day 14 after treatment initiation. However, the combination of *S. typhimurium* A1-R + o-rMETase was significantly more effective than TEM ($p < 0.0001$), o-rMETase alone ($p < 0.0001$), and TEM + o-rMETase ($p = 0.0051$). Only the combination of *S. typhimurium* A1-R + o-rMETase could regress the melanoma PDOX (Figs. 1B and 2).

3.2. Tumor targeting S. typhimurium A1-R

Confocal microscopy of bacteria cultured derived from the PDOX tumor showed *S. typhimurium* A1-R expressing green fluorescent protein (GFP) could directly target and proliferate in the melanoma PDOX and more *S. typhimurium* A1-R was growing in the tumor by day 14 than on day 5 (Fig. 3).

3.3. Body weight

We measured the body weight in each treatment group. Body weight loss was observed only in the TEM + rMETase combination group. However, there was no significant difference in body weight between any group (Fig. 4A). There were no animal deaths in any group.

3.4. Tumor histology

We also observed the tissue histology in each treatment group. Histologically, the untreated control tumor mainly comprised viable cells. In contrast, tumors treated with the *S. typhimurium* A1-R + rMETase combination showed extensive necrosis (Fig. 4B).

4. Discussion

TEM has been widely used as first-line chemotherapy for BRAF-V600E-negative melanoma, however with limited response [35–39]. In a previous study, we showed that this melanoma PDOX had MET dependency and the TEM + rMETase combination was significantly more effective than TEM alone [12]. However, this combination therapy could only inhibit tumor growth and did not regress the tumor, the “gold-standard” of mouse models of cancer

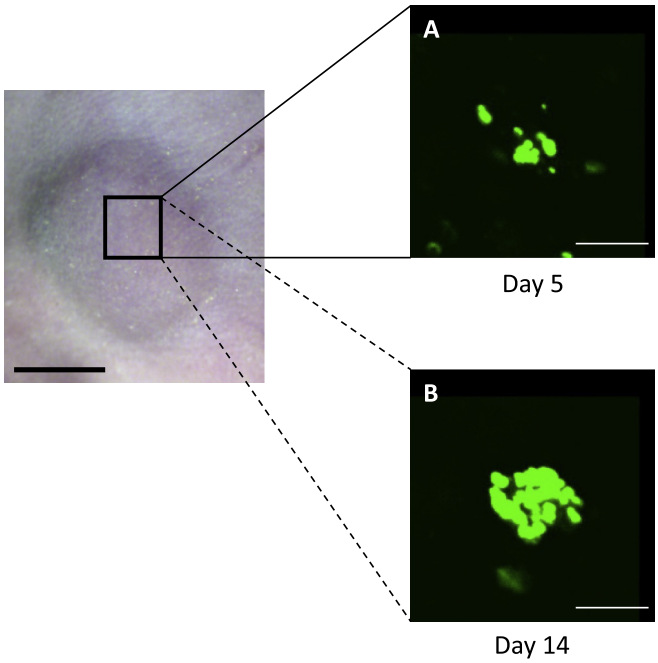

Fig. 3. Fluorescence imaging of *S. typhimurium* A1-R-GFP cultured from the melanoma PDOX. Confocal imaging with the FV1000 of *S. typhimurium* A1-R-GFP cultured from the melanoma PDOX. (A) Day 5, (B) Day 14. Black scale bar: 5 mm. White scale bar: 12.5 μm.

to predict clinical efficacy [40]. The gold standard was met in the present application by the combination of rMETase and *S. typhimurium* A1-R.

The PDOX models of melanoma should permit evaluation of therapeutic efficacy for individual patients based on the specific characteristics of the patient's tumor. We have developed the PDOX nude mouse model for all major cancers [5–14,18–24,27,28,41–48]. Our PDOX model has many advantages over subcutaneous-transplant models [49]. A PDOX model enables precise, individualized therapy, especially for recalcitrant diseases such as melanoma [8].

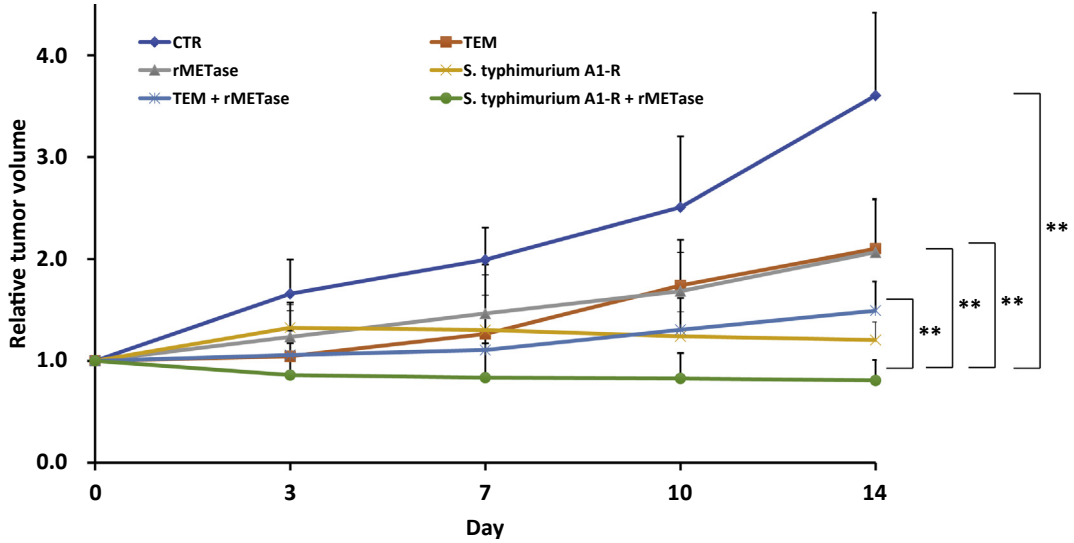

Fig. 2. Time course efficacy of treatment of the melanoma PDOX. Line graphs show relative tumor volume at each point relative to the initial tumor volume. **$p < 0.01$. Error bar: Mean ± SD.

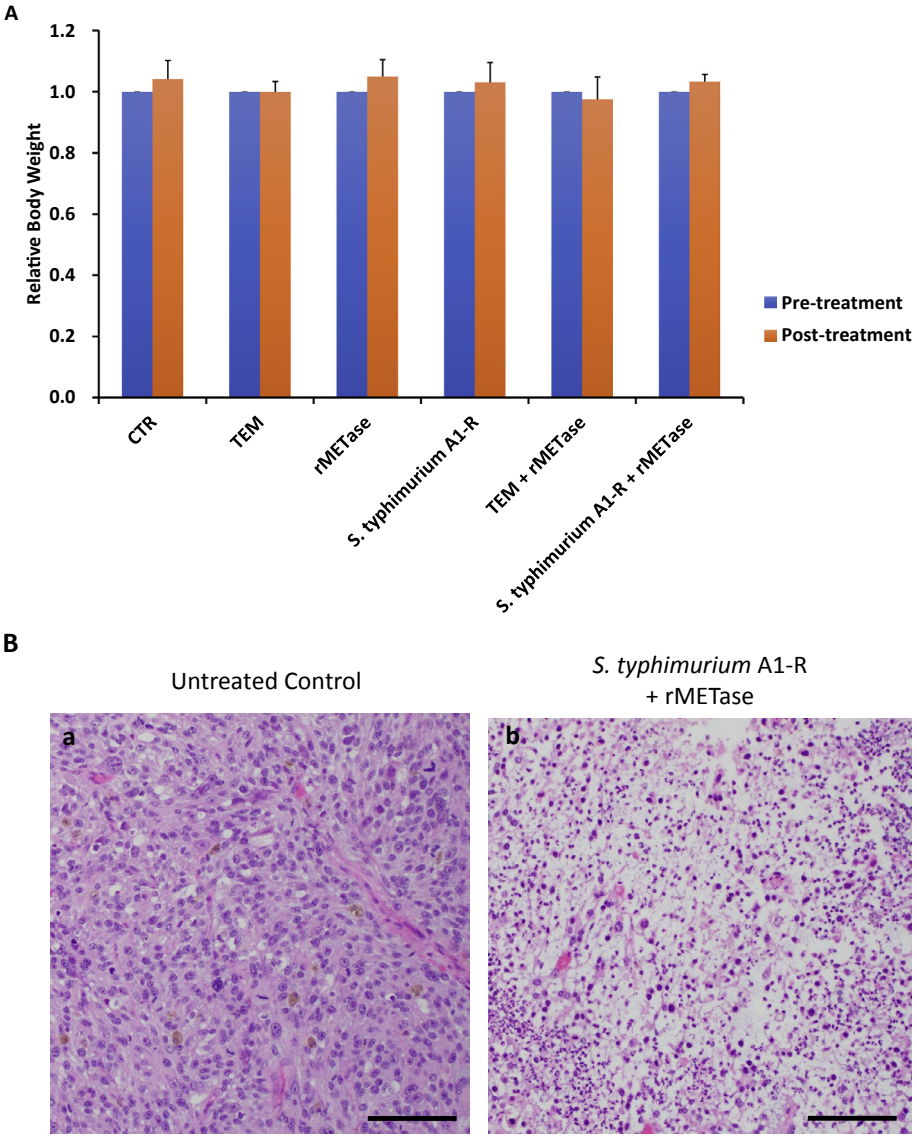

Fig. 4. Mouse body weight and tumor histology. (A) Effect of treatment on mouse body weight compared to the untreated control. Bar graphs show relative body weight at post-treatment relative to pre-treatment. (B) Tumor histology. (a) Untreated control. (b) Tumor treated with the combination of *S. typhimurium* A1-R + o-rMETase. Scale bars: 100 μm.

Altered cancer metabolism is currently being investigated for targets for effective novel therapeutics [50]. A very promising candidate target is the elevated MET of cancer cells, termed MET dependence. MET dependence may be the only known general metabolic defect in cancer [16,51,52]. MET dependence is observed when cancer cells selectively arrest upon MET restriction [15,51,52]. Tumor MET levels correlate with tumor size [14], further demonstrating the dependence of tumors on MET. MET dependence is due to MET overuse by cancer cells [15,51,53,54]. MET overuse can be observed in the clinic by the efficacy of [^{11}C] MET-PET imaging which gives a very strong signal, since the cancer tissue is taking up much more MET than the surrounding normal tissues [10]. MET restriction selectively arrests cancer cells in late S/G_2 of the cell cycle where the cancer cells become highly-sensitive to cytotoxic chemotherapy [55–60].

MET is sourced mainly from food. However, MET restriction through diets with low protein content does not allow the maintenance of good nutritional status. In addition, reduction of MET levels by dietary intervention is limited since MET is also sourced from the protein breakdown [51]. To more effectively target MET-dependence, we previously cloned *Psuedomonas putida* L-methionine α-deamino-γ-mercaptomethane lyase (recombinant methioninase [rMETase] [EC 4.4.1.11]) in *E. coli* for large-scale industrial production [61–67].

In the present study, we evaluated the efficacy of *S. typhimurium* A1-R alone and in combination with o-rMETase on the PDOX model of BRAF-V600E-negative melanoma. Only the combination of *S. typhimurium* A1-R + o-rMETase therapy could regress the tumor.

The present study has important clinical implications, as *S. typhimurium* A1-R combined with o-rMETase regressed a BRAF-V600E-negative melanoma in a PDOX model.

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Conflicts of interest

The authors declare that there are no potential conflicts of

interest.

References

[1] American Cancer Society, Cancer Facts and Figures 2018, American Cancer Society, Atlanta, Ga, 2018.
[2] Cancer Genome Atlas Network, Genomic classification of cutaneous melanoma, Cell 161 (2015) 1681–1696.
[3] G. Bollag, et al., Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma, Nature 467 (2010) 596–599.
[4] G.V. Long, J.S. Weber, J.R. Infante, et al., Overall survival and durable responses in patients with BRAF V600-mutant metastatic melanoma receiving dabrafenib combined with trametinib, J. Clin. Oncol. 34 (2016) 871–878.
[5] M. Yamamoto, M. Zhao, Y. Hiroshima, et al., Efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse model, PLoS One 11 (2016), e0160882.
[6] K. Kawaguchi, T. Murakami, B. Chmielowski, 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 7 (2016) 71737–71743.
[7] K. Kawaguchi, K. Igarashi, T. Murakami, 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 7 (2016) 85929–85936.
[8] K. Kawaguchi, K. Igarashi, T. Murakami, et al., Tumor-targeting *Salmonella typhimurium* A1-R sensitizes melanoma with a BRAF-V600E mutation to vemurafenib in a patient-derived orthotopic xenograft (PDOX) nude mouse model, J. Cell. Biochem. 118 (2017) 2314–2319.
[9] K. Kawaguchi, K. Igarashi, B. Chmielowski, et al., *Salmonella typhimurium* A1-R targeting of a chemotherapy resistant BRAF-V600E melanoma in a patient-derived orthotopic xenograft (PDOX) model is enhanced in combination with either vemurafenib or temozlomide, Cell Cycle 16 (2017) 1288–1294.
[10] R.M. Hoffman, Patient-derived orthotopic xenografts (PDOX) models of melanoma, Int. J. Mol. Sci. 18 (1875) 2017, https://doi.org/10.3390/ijms18091875.
[11] K. Kawaguchi, K. Igarashi, S. Li, et al., Combination treatment with recombinant methioninase enables temozolomide to arrest a BRAF V600E melanoma growth in a patient-derived orthotopic xenograft, Oncotarget 8 (2017) 85516–85525.
[12] K. Kawaguchi, K. Igarashi, S. Li, et al., Recombinant methioninase (rMETase) is an effective therapeutic for BRAF-V600E-negative as well as -positive melanoma in patient-derived orthotopic xenograft (PDOX) mouse models, Oncotarget 9 (2018) 915–923.
[13] K. Kawaguchi, Q. Han, S. Li, et al., Targeting methionine with oral recombinant methioninase (o-rMETase) arrests a patient-derived orthotopic xenograft (PDOX) model of BRAF-V600E mutant melanoma: implications for clinical cancer therapy and prevention, Cell Cycle 17 (2018) 356–361.
[14] K. Kawaguchi, Q. Han, S. Li, Y. Tan, et al., Intra-tumor L-methionine level highly correlates with tumor size in both pancreatic cancer and melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse models, Oncotarget 9 (2018) 11119–11125.
[15] R.M. Hoffman, R.W. Erbe, High *in vivo* rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 1523–1527.
[16] J.O. Mecham, D. Rowitch, C.D. Wallace, P.H. Stern, R.M. Hoffman, The metabolic defect of methionine dependence occurs frequently in human tumor cell lines, Biochem. Biophys. Res. Commun. 117 (1983) 429–434.
[17] R.M. Hoffman, Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis: a review and synthesis, Biochim. Biophys. Acta Rev. Canc 738 (1984) 49–87.
[18] T. Murakami, S. Li, Q. Han, et al., Recombinant methioninase effectively targets a Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model, Oncotarget 8 (2017) 35630–35638.
[19] K. Kawaguchi, K. Miyake, Q. Han, et al., Oral recombinant methioninase (o-rMETase) is superior to injectable rMETase and overcomes acquired gemcitabine resistance in pancreatic cancer, Canc. Lett. 432 (2018) 251–259.
[20] K. Igarashi, K. Kawaguchi, T. Kiyuna, et al., Effective metabolic targeting of human osteosarcoma cells in vitro and in orthotopic nude-mouse models with recombinant methioninase, Anticancer Res. 37 (2017) 4807–4812.
[21] K. Kawaguchi, K. Igarashi, S. Li, et al., Combination treatment with recombinant methioninase enables temozolomide to arrest a BRAF V600E melanoma growth in a patient-derived orthotopic xenograft, Oncotarget 8 (2017) 85516–85525.
[22] K. Igarashi, K. Kawaguchi, S. Li, et al., Recombinant methioninase in combination with DOX overcomes first-line DOX resistance in a patient-derived orthotopic xenograft nude-mouse model of undifferentiated spindle-cell sarcoma, Canc. Lett. 417 (2018) 168–173.
[23] K. Igarashi, S. Li, Q. Han, et al., Growth of a doxorubicin-resistant undifferentiated spindle-cell sarcoma PDOX is arrested by metabolic targeting with recombinant methioninase, J. Cell. Biochem. 119 (2018) 3537–3544.
[24] Igarashi, K., Kawaguchi, K., Kiyuna, T., et al. 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.
[25] Igarashi, K., Kawaguchi, K., Li, S., et al. Recombinant methioninase combined with doxorubicin (DOX) regresses a DOX-resistant synovial sarcoma in a patient-derived orthotopic xenograft (PDOX) mouse model. Oncotarget, 9(27):19263–19272.
[26] Kawaguchi, K., Miyake, K., Han, Q., et al. Targeting altered cancer methionine metabolism with recombinant methioninase (rMETase) overcomes partial gemcitabine-resistance and regresses a patient-derived orthotopic xenograft (PDOX) nude mouse model of pancreatic cancer. Cell Cycle, 17(7):868–873.
[27] M. Zhao, M. Yang, E. Baranov, et al., Spatial-temporal imaging of bacterial infection and antibiotic response in intact animals, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 9814–9818.
[28] M. Zhao, M. Yang, X.-M. Li, P. Jiang, et al., Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing *Salmonella typhimurium*, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 755–760.
[29] M. Zhao, M. Yang, H. Ma, X. Li, X. Tan, S. Li, Z. Yang, R.M. Hoffman, Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice, Canc. Res. 66 (2006) 7647–7652.
[30] Y. Hiroshima, M. Zhao, A. Maawy, et al., Efficacy of *Salmonella typhimurium* A1-R versus chemotherapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX), J. Cell. Biochem. 115 (2014) 1254–1261.
[31] K. Igarashi, K. Kawaguchi, T. Kiyuna, et al., Tumor-targeting *Salmonella typhimurium* A1-R is a highly effective general therapeutic for undifferentiated soft tissue sarcoma in patient-derived orthotopic xenograft (PDOX) nude-mouse models, Biochem. Biophys. Res. Commun. 497 (2018) 1055–1061.
[32] Y. Hiroshima, A. Maawy, C.A. Metildi, 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. 24 (2014) 241–247.
[33] Y. Tan, M. Xu, X.-Z. Tan, X.-Y. Tan, et al., Overexpression and large-scale production of recombinant l-methionine-α-deamino-γ-mercaptomethane-lyase for novel anticancer therapy, Protein Expr. Purif. 9 (1997) 233–245.
[34] A. Uchugonova, J. Duong, N. Zhang, K. König, R.M. Hoffman, The bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle, J. Cell. Biochem. 112 (2011) 2046–2050.
[35] P.B. Chapman, A. Hauschild, C. Robert, et al., Improved survival with vemurafenib in melanoma with BRAF V600E mutation, N. Engl. J. Med. 364 (2011) 2507–2516.
[36] H. Tang, Y. Wang, L.K. Chlewicki, et al., Facilitating T Cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade, Canc. Cell 29 (2016) 285–296.
[37] A.A. Brożyna, W. Jóźwicki, K. Roszkowski, J. Filipiak, A.T. Slominski, Melanin content in melanoma metastases affects the outcome of radiotherapy, Oncotarget 7 (2016), 17844–1785.
[38] A.T. Slominski, J.A. Carlson, Melanoma resistance: a bright future for academicians and a challenge for patient advocates, Mayo Clin. Proc. 89 (2014) 429–433.
[39] L.E. Flaherty, M. Othus, M.B. Atkins, et al., 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-risk melanoma—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 (2014) 3771–3778.
[40] Coleman, W.B., Tsongalis, G.J., Series edsR.M. Hoffman (Ed.), Patient-derived Mouse Models of Cancer. Molecular and Translational Medicine, Springer Intl. Publishing AG, 2017. ISSN:2197–7852.
[41] X. Fu, J.M. Besterman, A. Monosov, R.M. Hoffman, Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 9345–9349.
[42] C.A. Metildi, S. Kaushal, G.A. Luiken, M.A. Talamini, R.M. Hoffman, M. Bouvet, 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 (2014) 451–458.
[43] T. Furukawa, T. Kubota, M. Watanabe, M. Kitajima, X. Fu, R.M. Hoffman, 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. Canc. 53 (1993) 608–612.
[44] Y. Hiroshima, A.A. Maawy, M.H. Katz, J.B. Fleming, M. Bouvet, I. Endo, R.M. Hoffman, 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 (2015) 311–315.
[45] Y. Hiroshima, M. Zhao, Y. Zhang, et al., Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice, PLoS One 10 (2015), e0134324.
[46] K. Miyake, T. Murakami, T. Kiyuna, et al., The combination of temozolomide-irinotecan regresses a doxorubicin-resistant patient-derived orthotopic xenograft (PDOX) nude-mouse model of recurrent Ewing's sarcoma with a *FUS-ERG* fusion and CDKN2A deletion: direction for third-line patient therapy, Oncotarget 8 (2017) 103129–103136.
[47] K. Miyake, K. Kawaguchi, M. Miyake, et al., Tumor-targeting Salmonella typhimurium A1-R suppressed an imatinib-resistant gastrointestinal stromal tumor with c-kit exon 11 and 17 mutations, Heliyon 4 (6) (2018), e00643.
[48] K. Kawaguchi, K. Igarashi, K. Miyake, et al., MEK inhibitor trametinib in combination with gemcitabine regresses a patient-derived orthotopic

xenograft (PDOX) pancreatic cancer nude mouse model, Tissue Cell 52 (2018) 124–128.
[49] R.M. Hoffman, Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts, Nat. Rev. Canc. 15 (2015) 451–452.
[50] L.D. Chong, Exploiting cancer metabolism, Science 355 (2017) 1036.
[51] R.M. Hoffman, Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey, Expet Opin. Biol. Ther. 15 (2015) 21–31.
[52] Y. Tan, M. Xu, R.M. Hoffman, Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells *in vitro*, Anticancer Res. 30 (2010) 1041–1046.
[53] D.W. Coalson, J.O. Mecham, P.H. Stern, R.M. Hoffman, Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine dependent cancer cells, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 4248–4251.
[54] P.H. Stern, R.M. Hoffman, Elevated overall rates of transmethylation in cell lines from diverse human tumors, In Vitro – Rapid Commun. Cell Biol. 20 (1984) 663–670.
[55] R.M. Hoffman, S.J. Jacobsen, Reversible growth arrest in simian virus 40-transformed human fibroblasts, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 7306–7310.
[56] P.H. Stern, R.M. Hoffman, Enhanced *in vitro* selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect, J. Natl. Cancer Inst. 76 (1986) 629–639.
[57] H. Guo, V. Lishko, H. Herrera, A. Groce, T. Kubota, R.M. Hoffman, Therapeutic tumor-specific cell-cycle block induced by methionine starvation *in vivo*, Canc. Res. 53 (1993) 5676–5679.
[58] H.Y. Guo, H. Herrera, R.M. Hoffman, Unchecked DNA synthesis and blocked cell division induced by methionine deprivation in a human prostate cancer cell line, In Vitro Cell. Dev. Biol. 29A (1993) 359–361.
[59] S. Yano, S. Li, Q. Han, Y. Tan, M. Bouvet, T. Fujiwara, R.M. Hoffman, Selective methioninase-induced trap of cancer cells in S/G_2 phase visualized by FUCCI imaging confers chemosensitivity, Oncotarget 5 (2014) 8729–8736.
[60] S. Yano, K. Takehara, M. Zhao, Y. Tan, et al., 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 (2016) 1715–1723.
[61] Y. Tan, M. Xu, X.-Z. Tan, X.-Y. Tan, et al., Overexpression and large-scale production of recombinant l-methionine-α-deamino-γ-mercaptomethane-lyase for novel anticancer therapy, Protein Expr. Purif. 9 (1997) 233–245.
[62] T. Takakura, T. Ito, S. Yagi, Notsu, et al., High-level expression and bulk crystallization of recombinant l-methionine γ-lyase, an anticancer agent, Appl. Microbiol. Biotechnol. 70 (2006) 183–192.
[63] T. Takakura, A. Takimoto, Y. Notsu, et al., Physicochemical and pharmacokinetic characterization of highly potent recombinant l-methionine γ-lyase conjugated with polyethylene glycol as an antitumor agent, Canc. Res. 66 (2006) 2807–2814.
[64] D. Kudou, S. Misaki, M. Yamashita, et al., Structure of the antitumour enzyme l-methionine γ-lyase from *Pseudomonas putida* at 1.8Å resolution, J. Biochem. 141 (2007) 535–544.
[65] T. Takakura, K. Mitsushima, S. Yagi, et al., Assay method for antitumor l-methionine γ-lyase: comprehensive kinetic analysis of the complex reaction with l-methionine, Anal. Biochem. 327 (2004) 233–240.
[66] T. Yoshioka, T. Wada, N. Uchida, et al., Anticancer efficacy *in vivo* and *in vitro*, synergy with 5-fluorouracil, and safety of recombinant methioninase, Canc. Res. 58 (1998) 2583–2587.
[67] A. Uchugonova, J. Duong, N. Zhang, K. König, R.M. Hoffman, The bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle, J. Cell. Biochem. 112 (2011) 2046–2050.