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Recombinant Oral Methioninase (o-rMETase) Combined With Oxaliplatinum Plus 5-Fluorouracil Improves Survival of Mice With Massive Colon-Cancer Peritoneal Carcinomatosis

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Abstract. *Background/Aim: The present study aimed to determine if oral methioninase (o-rMETase) combined with oxaliplatinum (OXA) plus 5-fluorouracil (5-FU) increases survival of mice with peritoneal-carcinomatosis formed from HCT-116 green fluorescent protein (GFP)-expressing colon-cancer cells implanted intra-peritoneally in nude mice. Materials and Methods: HCT-116-GFP human colon-cancer cells (2×10^6) were injected intraperitoneally in athymic nude mice. Forty-five HCT-116-GFP colon-cancer peritoneal-carcinomatosis nude-mouse models were divided into the following groups: untreated control; combination of 5-FU (50 mg/kg, once a week), plus OXA (6 mg/kg, once a week); combination of 5-FU + OXA + o-rMETase (100 unit/day). Tumor growth was followed weekly using non-invasive GFP imaging for 3 weeks. At necropsy, tumor tissue was obtained. Frozen sections were made for fluorescence imaging. Tumor tissues were also stained with hematoxylin and eosin. The date of death of all mice was recorded. Results: o-rMETase combined with 5-FU + OXA significantly reduced peritoneal growth of the HCT-116 tumor compared to the untreated control or the combination 5-FU and OXA group. Histological analysis revealed extensive necrosis induced by the o-rMETase + 5-FU + OXA combination. The combination of 5-FU plus OXA and o-rMETase achieved significantly longer survival of the mice with peritoneal carcinomatosis compared to the control or combination of 5-FU plus OXA treatments. Conclusion: o-rMETase shows future clinical promise for increasing the survival of patients with peritoneal metastasis of colon cancer when combined with first-line treatment of this recalcitrant disease.*

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Key Words: Colon cancer, peritoneal metastasis, HCT-116, GFP, methionine addiction, Hoffman effect, methioninase, oral administration, 5-FU, oxaliplatinum, combination, synergy, Hoffman protocol.

Peritoneal metastasis of colon cancer is a recalcitrant disease, generally resistant to chemotherapy (1). Because of its poor prognosis, colon-cancer peritoneal carcinomatosis is a pre-terminal condition suitable only for palliative treatment. Currently first-line chemotherapy of colon-cancer peritoneal carcinomatosis comprises the combination of 5-fluorouracil (5-FU) together with oxaliplatinum (OXA).

The elevated methionine (MET) requirement of cancer cells is referred to as MET addiction or the Hoffman effect (2), which is analogous to the Warburg effect of excess glucose consumption by cancers (3). The Hoffman effect (2) is more pronounced than the Warburg effect (3) as seen by comparison of radioactive MET and deoxy-glucose positron emission tomography (PET) imaging (4). Methionine addiction is due to overuse of methionine for transmethylation reactions (5-11).

Recombinant methioninase (rMETase) (12) greatly inhibits the growth of all cancer cell types tested both *in vitro* and *in vivo* (12-18). Methionine restriction (MR) (19) is synergetic with chemotherapy such as 5-FU (20, 21) and cisplatinum (12, 22) due to selective cell-cycle arrest of methionine-restricted cancer cells in S/G_2 (17-19).

rMETase administered parenterally is highly effective on recalcitrant cancer (23-30). Oral rMETase (o-rMETase) is also highly effective, including when combined with 5-FU plus OXA on a colon-cancer patient-derived orthotropic xenograft (PDOX) mouse model and a peritoneal-surface metastatic mouse model to inhibit tumor growth (31-33).

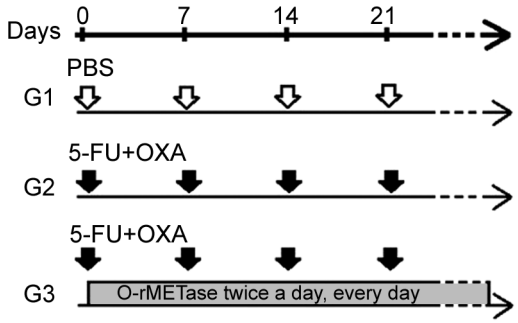

Figure 1. *Treatment schema. Group 1, Untreated control; Group 2, treatment with 5- fluorouracil (5-FU) + oxaliplatinum (OXA); Group 3, treatment with 5-FU+OXA + oral recombinant methioninase (o-rMETase).*

The present report demonstrates the survival efficacy of o-rMETase combined with 5-FU plus OXA on a colon-cancer peritoneal-metastasis nude-mouse model.

Materials and Methods

Mice and cells. Four to six-weeks-old athymic nu/nu nude mice were obtained from AntiCancer Inc. (San Diego, CA, USA). HCT-116 cells expressing green fluorescent protein (GFP) (AntiCancer Inc.) were thawed and cultured for one week and 2×10^6 cells in 100 μl phosphate-buffered saline (PBS) (Mediatech, Inc., Manassas, VA, USA) were injected to the right-lower-quadrant intraperitoneal space of each mouse. All animal experiments were conducted under National Institutes of Health (NIH) Assurance Number A3873-1 (33).

Production of rMETase. L-methionine-α-deamino-γ-mercaptomethane lyase (rMETase) (EC 4.4.1.11) was produced by fermentation of recombinant E. coli containing the METase gene from *P. putida* (AntiCancer, Inc.) and purified using a modified previous procedure (34). Pure methioninase was dissolved in PBS or water at 5 mg/ml.

Study design for treatment of colon-cancer peritoneal metastasis. Two weeks after cancer-cell intraperitoneal injection, colon-cancer peritoneal-metastasis models were randomized into three groups of fifteen mice. The first group served as a negative control and did not receive treatment. Mice in the second group were treated intraperitoneally once a week with 50 mg/kg 5-fluorouracil (5-FU), and 6 mg/kg oxaliplatinum (OXA). Mice in the third group received 5-FU, OXA, as in the second group, and 100 units/day of o-rMETase by gavage. All mice in the second and third groups were treated until they died (Figure 1).

Fluorescence imaging of peritoneal tumors. Mice were imaged non-invasively, weekly for peritoneal colon cancer. Fluorescence intensity of HCT-116-GFP tumors was measured and calculated using the UVP ibox® (Analytik Jena, Berlin, Germany) (Figure 2).

Tumor histology. At necropsy, tumor tissue was obtained, and frozen sections were made and observed under a confocal laser microscope (FV1000, Olympus Corp., Tokyo, Japan). The excitation wavelength for GFP fluorescence was 395 nm. Tumor tissues were embedded in melted paraffin wax and cut into thin sections (5 μm). The slides were stained with hematoxylin and counterstained with eosin.

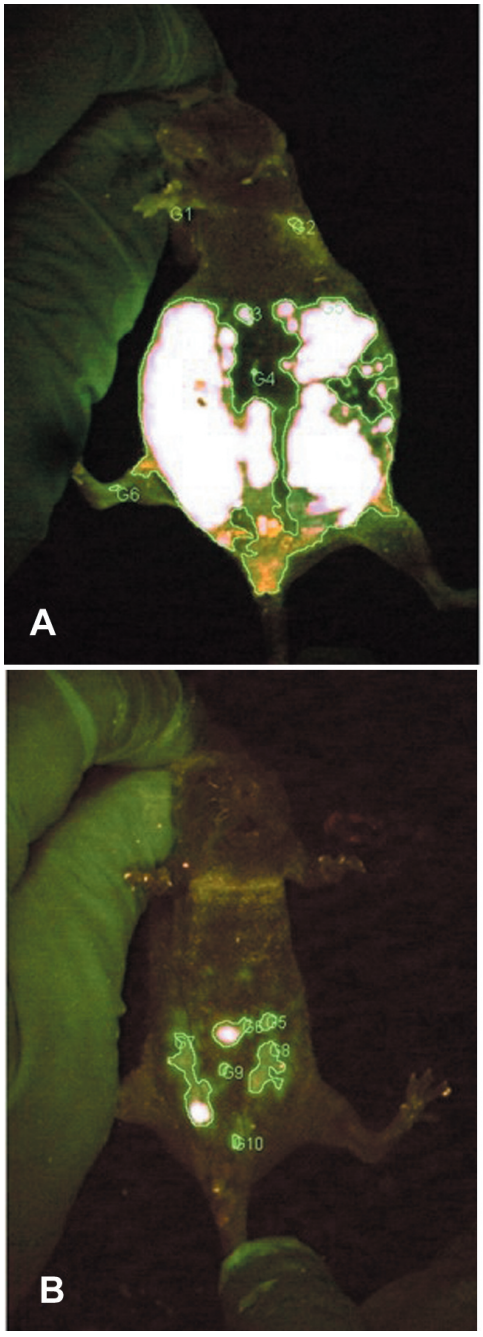

Figure 2. *Efficacy of treatment on colon-cancer peritoneal metastasis in nude mice implanted intraperitonelly with HTC-116-GFP human colon-cancer cells. Images were obtained 21 days after the start of treatment. (A) Green fluorescent protein (GFP) fluorescence image of an untreated control mouse. (B) GFP fluorescence image of a mouse treated with 5-fluorouracil (5-FU) + oxaliplatinum (OXA) + oral recombinant methioninase (o-rMETase).*

Statistical analysis. Significant differences for continuous variables were determined using the Student's *t*-test. *p*-Values of ≤0.05 were considered statistically significant. Mortality differences were evaluated by Kaplan-Meier analysis.

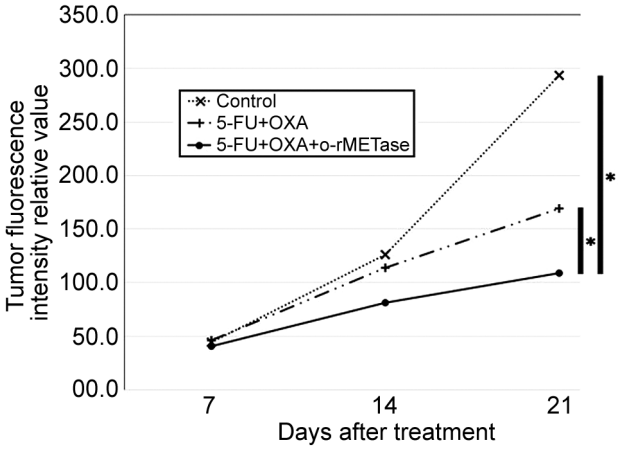

Figure 3. *Time-course of the tumor fluorescence intensity relative values of the treatment and control groups. 5-FU: 5-fluorouracil; OXA: oxaliplatinum; o-rMETase: oral recombinant methioninase. *p<0.05.*

Results

Efficacy of treatment on colon-cancer peritoneal metastasis. HCT-116-GFP tumor growth was measured by GFP fluorescence intensity over 21 days. The GFP fluorescence of the untreated HCT-116 tumor growing on the peritoneum was bright and extensive (Figure 2A) compared to mice treated with the combination of o-rMETase, 5-FU plus OXA (Figure 2B).

The fluorescence intensity of the combination of o-rMETase with 5-FU + OXA-treated mice was statistically-significantly lower than the control and 5-FU plus OXA treated mice over a 21-day treatment period ($p<0.05$) (Figure 3).

Tumor histology. Intense GFP fluorescence was observed in frozen sections of tumor from the peritoneum of an untreated mouse (Figure 4A). Hematoxylin and eosin (H&E) staining showed that the tumor tissue of the untreated control tumor mainly comprised viable carcinoma cells (Figure 4B). Only the combination of 5-FU, OXA and rMETase induced tumor necrosis (Figure 4C).

Survival. Kaplan-Meier analysis showed that survival of mice treated with the combination of o-rMETase with 5-FU + OXA was significantly extended compared to that of control or 5-FU + OXA-treated mice ($p<0.05$) (Figure 5). The mean survival time for all groups was: Group 1 (untreated control): 20.4 days; Group 2 (5-FU + OXA): 39.5 days; Group 3 (5-FU + OXA + rMETase): 44.6 days. Maximum survival for untreated control mice was 22 days; for mice treated with 5-FU + OXA, 47 days; and for the mice treated with o-rMETase + 5-FU + OXA, 60 days.

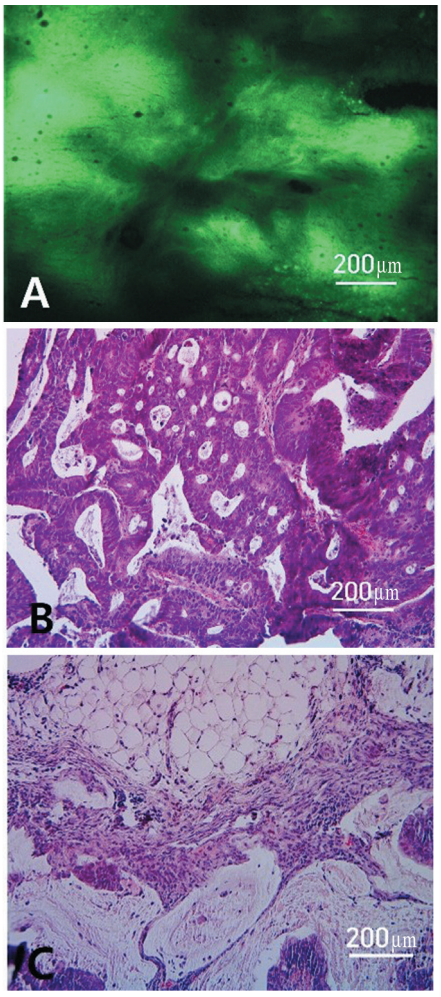

Figure 4. *Tumor histology. Tissue sections were obtained at necropsy. (A) Confocal-laser microscope image of a frozen section of the HCT-116-green fluorescent protein (GFP) tumor growing on the peritoneal surface of an untreated mouse. (B) Hematoxylin and eosin (H&E)-stained image from the HCT-116-GFP tumor growing on the peritoneum of an untreated mouse. (C) H&E-stained image from the HCT-116-GFP tumor growing on the peritoneum of a mouse treated with the combination of 5-fluorouracil (5-FU) + oxaliplatinum (OXA) + oral recombinant methioninase (o-rMETase).*

Discussion

All tested cancer types have been shown to be methionine addicted (15), and thus require a large amount of methionine (5-6, 35). Methionine restriction targets the methionine addiction of cancer cells and METase is very effective for methionine restriction (36). We originally discovered that methionine restriction combined with chemotherapy was synergistic (37), and is termed the Hoffman protocol (38). Our previous studies showed that o-rMETase combined with 5-FU plus OXA was effective on the growth of colon-cancer liver metastasis and peritoneal metastasis (31-33). In the

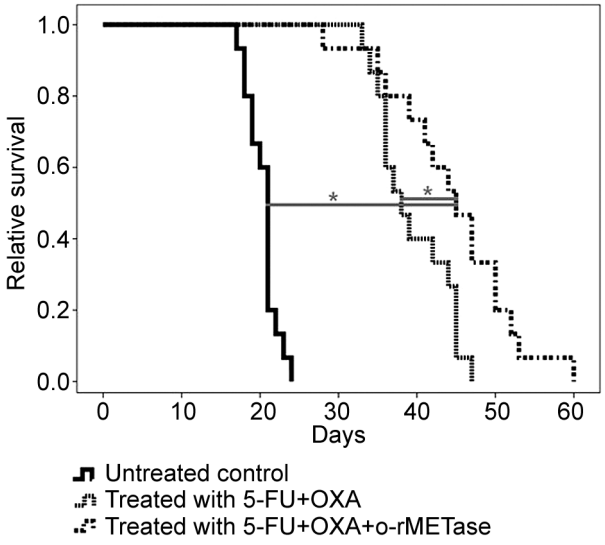

Figure 5. *Kaplan-Meier survival curves for overall survival of the treatment and control groups. 5-FU: 5-fluorouracil; OXA: oxaliplatinum; o-rMETase: oral recombinant methioninase. *p<0.05.*

present study, o-rMETase used in combination with 5-FU and OXA increased survival of a massive colon-cancer peritoneal-carcinomatosis HCT-116 nude-mouse model.

Clinical studies of o-rMETase both with and without chemotherapy have shown an effect on rectal cancer (39), pancreatic cancer (40) and prostate cancer (41-44) and breast-cancer metastasis (45). o-rMETase has little side effects. rMETase therapy has future clinical cancer use in combination with 5-FU plus OXA for recalcitrant colon-cancer peritoneal metastasis, because o-rMETase targets the fundamental basis of malignancy (5-11, 35, 46-51).

Conflicts of Interest

RMH is an unsalaried associate of AntiCancer. QH is an employee of AntiCancer. This research was supported in part by Grant no.2019-13 from the Kangdong Sacred Heart Hospital Fund and the Robert M. Hoffman Foundation for Cancer Research.

Authors' Contributions

Conception and design: MJK, JHP and RMH. QH provided the methioninase. Acquisition of data: MJK, JHP, QH and MB. Analysis and interpretation of data: MJK, QH, MB and JHP. Writing, review and revision of the manuscript: MJK, JHP and RMH.

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References

1 Jayne DG, Fook S, Loi C and Seow-Choen F: Peritoneal carcinomatosis from colorectal cancer. Br J Surg *89(12)*: 1545-1550, 2002. PMID: 12445064. DOI: 10.1046/j.1365-2168.2002.02274.x

2 Kaiser P: Methionine dependence of cancer. Biomolecules *10(4)*: 568, 2020. PMID: 32276408. DOI: 10.3390/biom10040568

3 Warburg O: The metabolism of carcinoma cells. The Journal of Cancer Research *9(1)*: 148-163, 2012. DOI: 10.1158/jcr.1925.148

4 Xu W, Gao L, Shao A, Zheng J and Zhang J: The performance of 11C-Methionine PET in the differential diagnosis of glioma recurrence. Oncotarget *8(53)*: 91030-91039, 2017. PMID: 29207622. DOI: 10.18632/oncotarget.19024

5 Hoffman RM: The wayward methyl group and the cascade to cancer. Cell Cycle *16(9)*: 825-829, 2017. PMID: 28318368. DOI: 10.1080/15384101.2017.1304330

6 Hoffman RM and Erbe RW: 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(5)*: 1523-1527, 1976. PMID: 179090. DOI: 10.1073/pnas.73.5.1523

7 Coalson DW, Mecham JO, Stern PH and Hoffman RM: Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells. Proc Natl Acad Sci USA *79(14)*: 4248-4251, 1982. PMID: 6289297. DOI: 10.1073/pnas.79.14.4248

8 Stern PH, Mecham JO, Wallace CD and Hoffman RM: Reduced free-methionine in methionine-dependent SV40-transformed human fibroblasts synthesizing apparently normal amounts of methionine. J Cell Physiol *117(1)*: 9-14, 1983. PMID: 6311851. DOI: 10.1002/jcp.1041170103

9 Stern PH and Hoffman RM: Elevated overall rates of transmethylation in cell lines from diverse human tumors. In Vitro *20(8)*: 663-670, 1984. PMID: 6500606. DOI: 10.1007/BF02619617

10 Yamamoto J, Inubushi S, Han Q, Tashiro Y, Sugisawa N, Hamada K, Aoki Y, Miyake K, Matsuyama R, Bouvet M, Clarke SG, Endo I and Hoffman RM: Linkage of methionine addiction, histone lysine hypermethylation, and malignancy. iScience *25(4)*: 104162, 2022. PMID: 35434545. DOI: 10.1016/j.isci.2022.104162

11 Hoffman RM, Coalson DW, Jacobsen SJ and Erbe RW: Folate polyglutamate and monoglutamate accumulation in normal and SV40-transformed human fibroblasts. J Cell Physiol *109(3)*: 497-505, 1981. PMID: 6274882. DOI: 10.1002/jcp.1041090316

12 Tan Y, Sun X, Xu M, Tan X, Sasson A, Rashidi B, Han Q, Tan X, Wang X, An Z, Sun FX and Hoffman RM: Efficacy of recombinant methioninase in combination with cisplatin on human colon tumors in nude mice. Clin Cancer Res *5(8)*: 2157-2163, 1999. PMID: 10473100.

13 Xin L, Cao J, Cheng H, Zeng F and Hu X: Stealth cationic liposomes modified with anti-CAGE single-chain fragment variable deliver recombinant methioninase for gastric carcinoma therapy. J Nanosci Nanotechnol *13(1)*: 178-183, 2013. PMID: 23646714. DOI: 10.1166/jnn.2013.6881

14 Xin L, Zhang HT, Yang WF, Li YF and Liu C: Evaluation of METase-pemetrexed-loaded PEG-PLGA nanoparticles modified with anti-CD133-scFV for treatment of gastric carcinoma. Biosci Rep *38(1)*: BSR20171001, 2018. PMID: 29229675. DOI: 10.1042/BSR20171001

15 Tan Y, Xu M and Hoffman RM: Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells in vitro. Anticancer Res *30(4)*: 1041-1046, 2010. PMID: 20530407.

16 Kreis W and Hession C: Biological effects of enzymatic deprivation of L-methionine in cell culture and an experimental tumor. Cancer Res *33(8)*: 1866-1869, 1973. PMID: 4720798.

17 Yano S, Takehara K, Zhao M, Tan Y, Han Q, Li S, Bouvet M, Fujiwara T and 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. PMID: 27152859. DOI: 10.1080/15384101.2016.1181240

18 Yano S, Li S, Han Q, Tan Y, Bouvet M, Fujiwara T and Hoffman RM: Selective methioninase-induced trap of cancer cells in S/G2 phase visualized by FUCCI imaging confers chemosensitivity. Oncotarget *5(18)*: 8729-8736, 2014. PMID: 25238266. DOI: 10.18632/oncotarget.2369

19 Hoffman RM and Jacobsen SJ: Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc Natl Acad Sci USA *77(12)*: 7306-7310, 1980. PMID: 6261250. DOI: 10.1073/pnas.77.12.7306

20 Hoshiya Y, Kubota T, Inada T, Kitajima M and Hoffman RM: Methionine-depletion modulates the efficacy of 5-fluorouracil in human gastric cancer in nude mice. Anticancer Res *17(6D)*: 4371-4375, 1997. PMID: 9494535.

21 Yoshioka T, Wada T, Uchida N, Maki H, Yoshida H, Ide N, Kasai H, Hojo K, Shono K, Maekawa R, Yagi S, Hoffman RM and Sugita K: Anticancer efficacy *in vivo* and *in vitro*, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res *58(12)*: 2583-2587, 1998. PMID: 9635582.

22 Hoshiya Y, Kubota T, Matsuzaki SW, Kitajima M and Hoffman RM: Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude mice. Anticancer Res *16(6B)*: 3515-3517, 1996. PMID: 9042214.

23 Kawaguchi K, Igarashi K, Li S, Han Q, Tan Y, Kiyuna T, Miyake K, Murakami T, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Unno M, Eilber FC and Hoffman RM: Combination treatment with recombinant methioninase enables temozolomide to arrest a BRAF V600E melanoma in a patient-derived orthotopic xenograft (PDOX) mouse model. Oncotarget *8(49)*: 85516-85525, 2017. PMID: 29156737. DOI: 10.18632/oncotarget.20231

24 Kawaguchi K, Igarashi K, Li S, Han Q, Tan Y, Miyake K, Kiyuna T, Miyake M, Murakami T, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Unno M, Eilber FC and Hoffman RM: 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(1)*: 915-923, 2017. PMID: 29416666. DOI: 10.18632/oncotarget.23185

25 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 and 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. PMID: 29374999. DOI: 10.1080/15384101.2018.1431596

26 Igarashi K, Li S, Han Q, Tan Y, Kawaguchi K, Murakami T, Kiyuna T, Miyake K, 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 and Hoffman RM: Growth of doxorubicin-resistant undifferentiated spindle-cell sarcoma PDOX is arrested by metabolic targeting with recombinant methioninase. J Cell Biochem *119(4)*: 3537-3544, 2018. PMID: 29143983. DOI: 10.1002/jcb.26527

27 Kawaguchi K, Miyake K, Han Q, Li S, Tan Y, Igarashi K, Lwin TM, Higuchi T, Kiyuna T, Miyake M, Oshiro H, Bouvet M, Unno M and Hoffman RM: 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, 2018. PMID: 29623758. DOI: 10.1080/15384101.2018.1445907

28 Kawaguchi K, Miyake K, Han Q, Li S, Tan Y, Igarashi K, Kiyuna T, Miyake M, Higuchi T, Oshiro H, Zhang Z, Razmjooei S, Wangsiricharoen S, Bouvet M, Singh SR, Unno M and Hoffman RM: Oral recombinant methioninase (o-rMETase) is superior to injectable rMETase and overcomes acquired gemcitabine resistance in pancreatic cancer. Cancer Lett *432*: 251-259, 2018. PMID: 29928962. DOI: 10.1016/j.canlet.2018.06.016

29 Higuchi T, Kawaguchi K, Miyake K, Han Q, Tan Y, Oshiro H, Sugisawa N, Zhang Z, Razmjooei S, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Chawla SP, Singh AS, Eilber FC, Singh SR, Tsuchiya H and Hoffman RM: Oral recombinant methioninase combined with caffeine and doxorubicin induced regression of a doxorubicin-resistant synovial sarcoma in a PDOX mouse model. Anticancer Res *38(10)*: 5639-5644, 2018. PMID: 30275182. DOI: 10.21873/anticanres.12899

30 Miyake K, Kiyuna T, Miyake M, Kawaguchi K, Zhang Z, Wangsiricharoen S, Razmjooei S, Oshiro H, Higuchi T, Li Y, Nelson SD, Murakami T, Hiroshima Y, Kumamoto T, Matsuyama R, Bouvet M, Singh SR, Chawla SP, Endo I and Hoffman RM: Gemcitabine combined with docetaxel precisely regressed a recurrent leiomyosarcoma peritoneal metastasis in a patient-derived orthotopic xenograft (PDOX) model. Biochem Biophys Res Commun *509(4)*: 1041-1046, 2019. PMID: 30660363. DOI: 10.1016/j.bbrc.2019.01.046

31 Oshiro H, Tome Y, Kiyuna T, Yoon SN, Lwin TM, Han Q, Tan Y, Miyake K, Higuchi T, Sugisawa N, Katsuya Y, Park JH, Zang Z, Razmjooei S, Bouvet M, Clary B, Singh SR, Kanaya F, Nishida K and Hoffman RM: Oral recombinant methioninase overcomes colorectal-cancer liver metastasis resistance to the combination of 5-fluorouracil and oxaliplatinum in a patient-derived orthotopic xenograft mouse model. Anticancer Res *39(9)*: 4667-4671, 2019. PMID: 31519565. DOI: 10.21873/anticanres.13648

32 Park JH, Han Q, Zhao M, Tan Y, Higuchi T, Yoon SN, Sugisawa N, Yamamoto J, Bouvet M, Clary B, Singh SR and Hoffman RM: Oral recombinant methioninase combined with oxaliplatinum and 5-fluorouracil regressed a colon cancer growing on the peritoneal surface in a patient-derived orthotopic xenograft mouse model. Tissue Cell *61*: 109-114, 2019. PMID: 31759402. DOI: 10.1016/j.tice.2019.09.006

33 Park JH, Han Q, Zhao M, Tan Y, Higuchi T, Yoon SN, Sugisawa N, Yamamoto J, Bouvet M, Clary B, Singh SR and Hoffman RM: Oral recombinant methioninase combined with oxaliplatinum and

5-fluorouracil regressed a colon cancer growing on the peritoneal surface in a patient-derived orthotopic xenograft mouse model. Tissue Cell *61*: 109-114, 2019. PMID: 31759402. DOI: 10.1016/j.tice.2019.09.006

34 Tan Y, Xu M, Tan X, Tan X, Wang X, Saikawa Y, Nagahama T, Sun X, Lenz M and Hoffman RM: Overexpression and large-scale production of recombinant L-methionine-alpha-deamino-gamma-mercaptomethane-lyase for novel anticancer therapy. Protein Expr Purif *9(2)*: 233-245, 1997. PMID: 9056489. DOI: 10.1006/prep.1996.0700

35 Wang Z, Yip LY, Lee JHJ, Wu Z, Chew HY, Chong PKW, Teo CC, Ang HY, Peh KLE, Yuan J, Ma S, Choo LSK, Basri N, Jiang X, Yu Q, Hillmer AM, Lim WT, Lim TKH, Takano A, Tan EH, Tan DSW, Ho YS, Lim B and Tam WL: Methionine is a metabolic dependency of tumor-initiating cells. Nat Med *25(5)*: 825-837, 2019. PMID: 31061538. DOI: 10.1038/s41591-019-0423-5

36 Hoffman RM: Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. Expert Opin Biol Ther *15(1)*: 21-31, 2015. PMID: 25439528. DOI: 10.1517/14712598.2015.963050

37 Stern PH and Hoffman RM: Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. J Natl Cancer Inst *76(4)*: 629-639, 1986. PMID: 3457200. DOI: 10.1093/jnci/76.4.629

38 Masaki N, Han Q, Samonte C, Wu NF, Hozumi C, Wu J, Obara K, Kubota Y, Aoki Y, Bouvet M and Hoffman RM: Oral-recombinant methioninase in combination with rapamycin eradicates osteosarcoma of the breast in a patient-derived orthotopic xenograft mouse model. Anticancer Res *42(11)*: 5217-5222, 2022. PMID: 36288875. DOI: 10.21873/anticanres.16028

39 Kubota Y, Han Q, Hamada K, Aoki Y, Masaki N, Obara K, Tsunoda T and Hoffman RM: Long-term stable disease in a rectal-cancer patient treated by methionine restriction with oral recombinant methioninase and a low-methionine diet. Anticancer Res *42(8)*: 3857-3861, 2022. PMID: 35896248. DOI:10.21873/anticanres.15877

40 Kubota Y, Han Q, Hozumi C, Masaki N, Yamamoto J, Aoki Y, Tsunoda T and Hoffman RM: Stage IV pancreatic cancer patient treated with FOLFIRINOX combined with oral methioninase: a highly-rare case with long-term stable disease. Anticancer Res *42(5)*: 2567-2572, 2022. PMID: 35489727. DOI: 10.21873/anticanres.15734

41 Han Q and Hoffman RM: Lowering and stabilizing PSA levels in advanced-prostate cancer patients with oral methioninase. Anticancer Res *41(4)*: 1921-1926, 2021. PMID: 33813397. DOI: 10.21873/anticanres.14958

42 Yamamoto J, Han Q, Simon M, Thomas D and Hoffman RM: Methionine restriction: Ready for prime time in the cancer clinic? Anticancer Res *42(2)*: 641-644, 2022. PMID: 35093861. DOI: 10.21873/anticanres.15521

43 Han Q, Tan Y and Hoffman RM: Oral dosing of recombinant methioninase is associated with a 70% drop in PSA in a patient with bone-metastatic prostate cancer and 50% reduction in circulating methionine in a high-stage ovarian cancer patient. Anticancer Res *40(5)*: 2813-2819, 2020. PMID: 32366428. DOI: 10.21873/anticanres.14254

44 Han Q and Hoffman RM: Chronic treatment of an advanced prostate-cancer patient with oral methioninase resulted in long-term stabilization of rapidly rising PSA levels. In Vivo *35(4)*: 2171-2176, 2021. PMID: 34182494. DOI: 10.21873/invivo.12488

45 Kubota Y, Han Q, Masaki N, Hozumi C, Hamada K, Aoki Y, Obara K, Tsunoda T and Hoffman RM: Elimination of axillary-lymph-node metastases in a patient with invasive lobular breast cancer treated by first-line neo-adjuvant chemotherapy combined with methionine restriction. Anticancer Res 42(12): 5819-5823, 2022. PMID: 36456116. DOI: 10.21873/anticanres.16089

46 Yamamoto J, Han Q, Inubushi S, Sugisawa N, Hamada K, Nishino H, Miyake K, Kumamoto T, Matsuyama R, Bouvet M, Endo I and Hoffman RM: Histone methylation status of H3K4me3 and H3K9me3 under methionine restriction is unstable in methionine-addicted cancer cells, but stable in normal cells. Biochem Biophys Res Commun *533(4)*: 1034-1038, 2020. PMID: 33019978. DOI: 10.1016/j.bbrc.2020.09.108

47 Aoki Y, Han Q, Tome Y, Yamamoto J, Kubota Y, Masaki N, Obara K, Hamada K, Wang JD, Inubushi S, Bouvet M, Clarke SG, Nishida K and Hoffman RM: Reversion of methionine addiction of osteosarcoma cells to methionine independence results in loss of malignancy, modulation of the epithelial-mesenchymal phenotype and alteration of histone-H3 lysine-methylation. Front Oncol *12*: 1009548, 2022. PMID: 36408173. DOI: 10.3389/fonc.2022.1009548

48 Yamamoto J, Aoki Y, Inubushi S, Han Q, Hamada K, Tashiro Y, Miyake K, Matsuyama R, Bouvet M, Clarke SG, Endo I and Hoffman RM: Extent and instability of trimethylation of histone H3 lysine increases with degree of malignancy and methionine addiction. Cancer Genomics Proteomics *19(1)*: 12-18, 2022. PMID: 34949655. DOI: 10.21873/cgp.20299

49 Hoffman RM, Jacobsen SJ and Erbe RW: Reversion to methionine independence by malignant rat and SV40-transformed human fibroblasts. Biochem Biophys Res Commun 82(1): 228-234, 1978. PMID: 208554. DOI: 10.1016/0006-291x(78)90600-9

50 Hoffman RM, Jacobsen SJ and Erbe RW: Reversion to methionine independence in simian virus 40-transformed human and malignant rat fibroblasts is associated with altered ploidy and altered properties of transformation. Proc Natl Acad Sci 76(3): 1313, 1979. PMID: 220612. DOI: 10.1073/pnas.76.3.1313

51 Breillout F, Antoine E and Poupon MF: Methionine dependency of malignant tumors: a possible approach for therapy. J Natl Cancer Inst 82(20): 1628-1632, 1990. PMID: 2213904. DOI: 10.1093/jnci/82.20.1628

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