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Recombinant-methioninase-producing *Escherichia coli* Instilled in the Microbiome Inhibits Triple-negative Breast Cancer in an Orthotopic Cell-line Mouse Model

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Abstract. *Background/Aim: Methionine restriction by diet and recombinant methioninase (rMETase) are effective for cancer therapy by themselves or combined with chemotherapy drugs. We previously showed that oral administration of rMETase-producing Escherichia coli JM109 (E. coli JM109-rMETase) can be installed in the mouse microbiome and inhibit colon-cancer growth in a syngeneic mouse model. In the present report, we investigated the efficacy of oral administration of E. coli JM109-rMETase in an orthotopic triple-negative breast cancer (TNBC) cell-line mouse model. Materials and Methods: First, we established orthotopic 4T1 mouse triple-negative breast cancer on an abdominal mammary gland in female athymic nu/nu nude mice aged 4-6 weeks. After tumor growth, 15 mice were divided into three groups of 5. Group 1 was administered phosphate-buffered saline (PBS) orally by gavage twice daily as a control; Group 2 was administered non-recombinant E. coli JM109 competent cells orally by gavage twice daily as a control; Group 3 was administered E. coli JM109-rMETase cells by gavage twice daily for two weeks. Tumor size was measured with calipers twice per week. On day 15, blood methionine level was examined using an HPLC method. Results: Oral administration of E. coli JM109-rMETase inhibited 4T1 TNBC growth significantly compared to the PBS and E. coli JM109 control groups. On day 15, the blood methionine level was significantly lower in the mice administered E. coli JM109-rMETase than in the PBS control. Conclusion: E. coli JM109-rMETase lowered blood methionine levels and inhibited TNBC growth in an orthotopic cell-line mouse model, suggesting future clinical potential against a highly recalcitrant cancer.*

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Key Words: Methionine addiction, Hoffman effect, *Escherichia coli*, recombinant methioninase, triple negative breast cancer, 4T1 cell-line, orthotopic, mouse model, tumor-growth inhibition, methionine depletion.

Methionine addiction is a fundamental and general hallmark of cancer, termed the Hoffman effect (1-4). Methionine addiction is due to excess transmethylation reactions in cancer cells. Unlike normal cells, cancer cells cannot survive without exogenous methionine (5-11).

We have reported the efficacy of recombinant methioninase (rMETase) for various cancer types in vitro, in vivo, and in the clinic (12). Oral administration of rMETase (o-rMETase), is highly effective in mouse models of cancer and is promising in the clinic (13-19). We previously reported the efficacy of o-rMETase for triple-negative breast cancer (TNBC) using a patient-derived orthotopic xenograft model (20-22).

Recently, we showed the efficacy of rMETase-producing *E. coli JM109* (*E. coli JM109*-rMETase) administered orally against the mouse colon-cancer cell line MC38 in a syngeneic mouse model (23). The present report shows that oral administration of *E. coli JM109*-rMETase can be instilled in the mouse gut and inhibit breast-cancer growth.

Breast cancer is the most common type of cancer and the second-leading cause of death among women in the United States (24). Breast cancer has three main subtypes: hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-positive, and TNBC, according to molecular

markers, such as estrogen receptor (ER), progesterone receptor (PR), and HER2. TNBC has a worse prognosis compared to HR-positive or HER2-positive breast cancer (25, 26). The standard treatment for metastatic or recurrent TNBC is chemotherapy with single-agent taxanes or anthracyclines. Recently, the efficacy of the combination of chemotherapy with an immune checkpoint inhibitor, such as atezolizumab or pembrolizumab has shown a median overall survival of less than 23 months (27, 28).

In the present report, we examined the efficacy of *E. coli JM109*-rMETase on the mouse TNBC cell line 4T1 using an orthotopic cell-line mouse model.

Materials and Methods

E. coli JM109-rMETase culture. The host strain for the expression of rMETase was *E. coli JM109*. The plasmid pATG3131, which contains the tetracycline (TC)-resistance gene, was used to clone the rMETase gene from *P. putida* into *E. coli JM109* (29, 30). The resultant *E. coli JM109*-rMETase was pre-cultured in 5 ml Luria-Bertani (LB) liquid medium for 8 h at 37˚C with TC (32 μg/ml). The preculture broth was placed in 400 ml culture medium with TC (32 μg/ml) and grown overnight. To induce the expression of rMETase, isopropyl-D-thiogalactopyranoside (IPTG) was administered at a final concentration of 0.3 mM for 4 h at 37˚C. To estimate the concentration of live *E. coli JM109*-rMETase in the medium, the optical density (OD) at 600 nm was used. An OD_{600} of 0.7 corresponds to approximately 1.0×10^9 colony-forming units (CFU)/ml of *E. coli JM109*-rMETase, correlating to manually counting colonies. Harvested *E. coli JM109*-rMETase was diluted with phosphate-buffered saline (PBS), 20% glycerin, and stored at 80˚C before being administered to mice. Similar to *E. coli JM109*-rMETase, *E. coli JM109* competent cells, not treated with TC or IPTG, were prepared (23).

Mice. The present study used female athymic *nu/nu* nude mice (AntiCancer Inc., San Diego, CA, USA) that were 4-6 weeks old. The mice were kept in a barrier facility with a HEPA-filtered rack and 12-h light/dark cycle. During this experiment, mice were fed autoclaved laboratory rodent food. The AntiCancer Institutional Animal Care and Use Committee approved the study. Every experiment followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. 2.0 criteria (31).

Cell culture. High-glucose Dulbecco's-modified Eagle's medium (DMEM), containing 10% fetal bovine serum (FBS) and 100 IU/ml penicillin/streptomycin was used to cultivate the 4T1 mouse triple-negative breast cancer cell line at 37˚C in a humid environment containing 5% carbon dioxide.

Establishment of orthotopic tumors. Twenty female athymic *nu/nu* nude mice, aged 4-6 weeks were given an orthotopic injection of 10^6 4T1 cells in an abdominal mammary gland. One week after injection, orthotopic tumors were established.

Treatment study design. Mice implanted orthotopically with 4T1 were randomized into three groups of five mice each: Group 1: Untreated control given 100 μl phosphate buffered saline (PBS) orally twice daily (9 a.m. and 5 p.m.) for 14 days; Group 2: *E. coli JM109* competent cells (10^{10}/100 μl) were administered orally by gavage twice daily (9 a.m. and 5 p.m.) for 14 days: Group 3: *E. coli JM109*-rMETase cells (10^{10}/100 μl) were administered orally by gavage twice daily (9 a.m. and 5 p.m.) for 14 days. To prevent plasmid shedding in Group 3, TC (0.5 g/l) was added to the drinking water of the mice. TC was added to Group I as a control. IPTG was only administered to Group 3 in order to induce the production of methioninase by *E. coli JM109*-rMETase in the gastrointestinal tract of the mice. Tumor volume and body weight were measured twice a week during the treatment. Tumor volume (mm^3) was calculated with the following formula: Tumor volume (mm^3)=length (mm) × width (mm) × width (mm) × 1/2.

Measurement of plasma-methionine concentration. Mouse blood samples were collected through tail bleeding between 9 and 10 a.m. on day 15 for Group 1 and Group 3. The quantification of methionine in the plasma was conducted using pre-column derivatization, followed by high-performance liquid chromatography (HPLC) separation, following a previously-established protocol (32).

Statistics. Statistical analyses were carried out using GraphPad Prism 9.4.0 software (GraphPad Software, Inc., San Diego, CA, USA). The unpaired *t*-test or Tukey-Kramer post hoc test were used for the parametric comparison between groups. The data are presented as the mean plus-minus the standard deviation. Significance was attributed to *p*-values that were less than or equal to 0.05.

Results

Antitumor efficacy of oral E. coli JM109-rMETase. The growth of 4T1 tumors in athymic *nu/nu* nude mice was significantly suppressed by *E. coli JM109*-rMETase when compared to the PBS-treated control or *E. coli JM109* competent cells that do not produce rMETase (p=0.0034 for the PBS control *vs*. *E. coli JM109*-rMETase; p=0.0113 for *E. coli JM109* competent-cells control *vs*. *E. coli JM109*-rMETase) (Figure 1).

Body-weight change. Figure 2 shows the body-weight change of each group. No significant change in any group confirms the tolerability of 10^{10}/100 μl *E. coli JM109* for nude mice.

Methionine level. On day 15, the mean methionine level of Group 1 and Group 3 was as follows: *E. coli JM109* competent-cells control group: 85.3 μM; *E. coli JM109*-rMETase group: 60.5 μM (p=0.0133), which is approximately a 30% reduction (Figure 3).

Discussion

We previously showed the efficacy of orally-administered *E. coli JM109*-rMETase against the mouse colon-cancer cell line MC38 in a syngeneic mouse model (23). The present study showed that *E. coli JM109*-rMETase is also effective against mouse TNBC. This bacterial therapy reduced blood methionine levels by approximately 30%, which is consistent with the efficacy of oral rMETase (33).

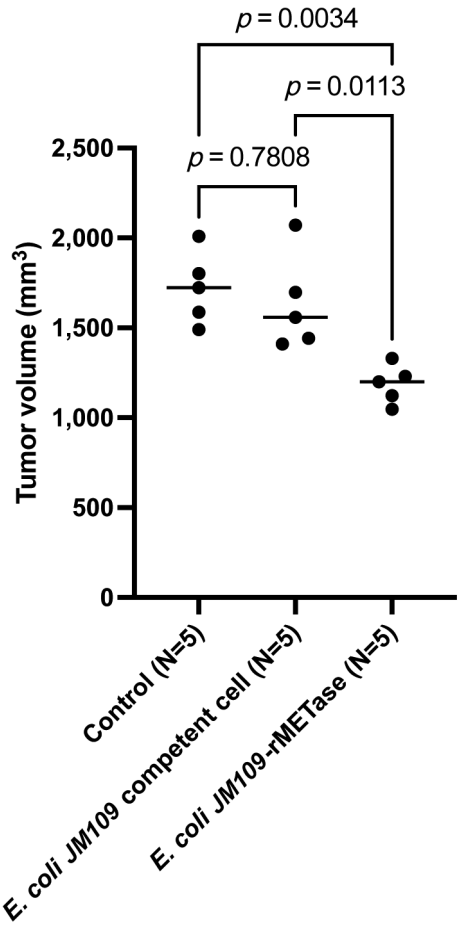

Figure 1. *Efficacy of recombinant methioninase (rMETase)-producing Escherichia coli JM109 (E. coli JM109-rMETase) against the 4T1 mouse triple negative breast cancer in an athymic nu/nu nude-mouse orthotopic model on day 15.*

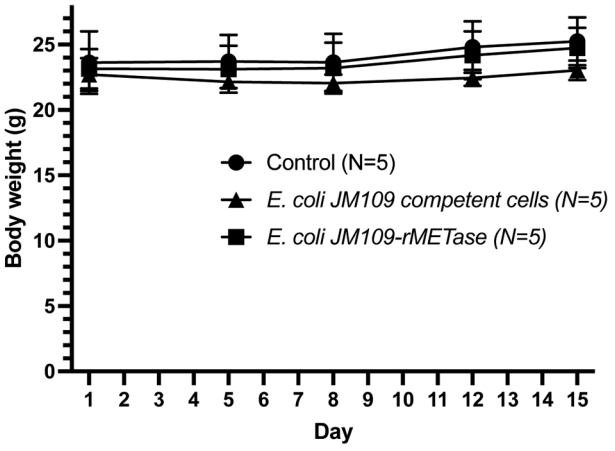

Figure 2. *Body-weight change during the treatment period. Data are shown as the mean±standard deviation.*

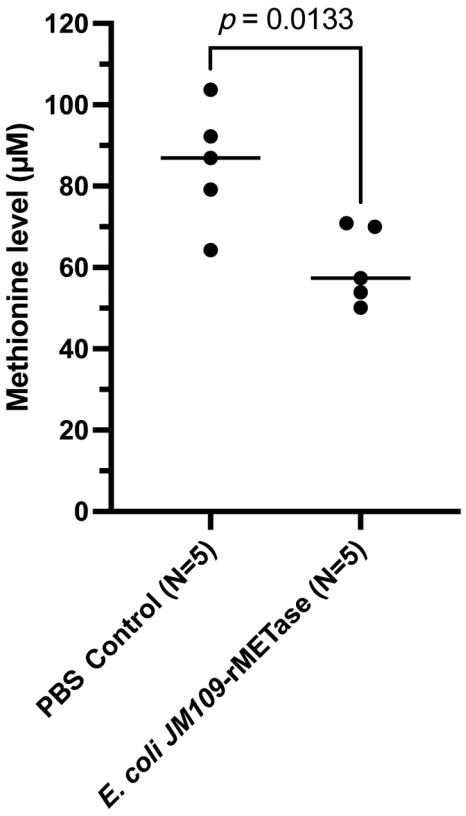

Figure 3. *Blood methionine level at day 15.*

E. coli JM109-rMETase acts in the gut, and is also instilled in the microbiome and produces rMETase due to IPTG in the drinking water (23). The rMETase produced by the bacteria degrades methionine. Also, deoxycholic acid from the liver may lyse the bacteria, thereby releasing methioninase into the gut. Since intestinal epithelial cells consume significant amounts of methionine, approximately 20-30% of methionine is metabolized during absorption (34). Therefore, it is reasonable to target the intestine for methionine restriction with *E. coli JM109*-rMETase.

In a recent study, the genetically modified strain of *E. coli* Nissle was employed to metabolize methionine through the methionine decarboxylase pathway (35). The findings revealed a decrease of 32% in blood methionine levels in mice, and 25-26% in humans. This concept is a methionine-consuming recombinant pro-biotic engineered with methionine decarboxylase. This pro-biotic was used to treat homocystinuria by lowering the amount of methionine in the blood of humans.

In conclusion, bacterial therapy using *E. coli JM109*-rMETase showed growth-inhibitory efficacy against a mouse

TNBC 4T1, in nude mice. As demonstrated by *E. coli JM109*-rMETase in the present and previous reports (23), recent advances in manipulating the gut microbiome to reduce methionine indicate the future potential for treating recalcitrant cancer. Methionine-restriction therapy is effective since it targets the fundamental basis of cancer, methionine addiction (1-11, 36-42).

The US National Cancer Institute has been allocated $160 billion since 1971, yet the mean survival of TNBC patients is less than 23 months (27, 28). Methionine-restriction therapy should increase TNBC survival.

Conflicts of Interest

The Authors declare no competing interests regarding this work.

Authors' Contributions

YK and RMH wrote the article. QH, SM, KM, MB and TT reviewed the article.

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References

1 Hoffman RM, Erbe RW: High *in vivo* rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci USA 73(5): 1523-1527, 1976. DOI: 10.1073/pnas.73.5.1523

2 Coalson DW, Mecham JO, Stern PH, 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. DOI: 10.1073/pnas.79.14.4248

3 Stern PH, Mecham JO, Wallace CD, 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. DOI: 10.1002/jcp.1041170103

4 Kaiser P: Methionine Dependence of Cancer. Biomolecules 10(4): 568, 2020. DOI: 10.3390/biom10040568

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

6 Judde JG, Ellis M, Frost P: Biochemical analysis of the role of transmethylation in the methionine dependence of tumor cells. Cancer Res 49: 4859-4865, 1989.

7 Yamamoto J, Han Q, Inubushi S, Sugisawa N, Hamada K, Nishino H, Miyake K, Kumamoto T, Matsuyama R, Bouvet M, Endo I, 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. DOI: 10.1016/j.bbrc.2020.09.108

8 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, Tam WL: Methionine is a metabolic dependency of tumor-initiating cells. Nat Med 25(5): 825-837, 2019. DOI: 10.1038/s41591-019-0423-5

9 Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM: The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem Biophys Res Commun 117(2): 429-434, 1983. DOI: 10.1016/0006-291X(83)91218-4

10 Stern PH, Wallace CD, Hoffman RM: Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines. J Cell Physiol 119(1): 29-34, 1984. DOI: 10.1002/jcp.1041190106

11 Tan Y, Xu M, Hoffman RM: Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells in vitro. Anticancer Res 30: 1041-1046, 2010.

12 Kubota Y, Han Q, Aoki Y, Masaki N, Obara K, Hamada K, Hozumi C, Wong ACW, Bouvet M, Tsunoda T, Hoffman RM: Synergy of combining methionine restriction and chemotherapy: The disruptive next generation of cancer treatment. Cancer Diagn Progn 3(3): 272-281, 2023. DOI: 10.21873/cdp.10212

13 Kawaguchi K, Han Q, Li S, Tan Y, Igarashi K, Kiyuna T, Miyake K, Miyake M, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Singh AS, Eckardt MA, Unno M, Eilber FC, Hoffman RM: Targeting methionine with oral recombinant methioninase (o-rMETase) arrests a patient-derived orthotopic xenograft (PDOX) model of BRAF-V600E mutant melanoma: implications for chronic clinical cancer therapy and prevention. Cell Cycle 17(3): 356-361, 2018. DOI: 10.1080/15384101.2017.1405195

14 Kawaguchi K, Han Q, Li S, Tan Y, Igarashi K, Murakami T, Unno M, Hoffman RM: Efficacy of recombinant methioninase (rMETase) on recalcitrant cancer patient-derived orthotopic xenograft (PDOX) mouse models: a review. Cells 8(5): 410, 2019. DOI: 10.3390/cells8050410

15 Han Q, Tan Y, 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. DOI: 10.21873/anticanres.14254

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

17 Kubota Y, Han Q, Hozumi C, Masaki N, Yamamoto J, Aoki Y, Tsunoda T, 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. DOI: 10.21873/anticanres.15734

18 Kubota Y, Han Q, Masaki N, Hozumi C, Hamada K, Aoki Y, Obara K, Tsunoda T, 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. DOI: 10.21873/anticanres.16089

19 Kubota Y, Han Q, Hamada K, Aoki Y, Masaki N, Obara K, Tsunoda T, 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. DOI: 10.21873/anticanres.15877

20 Lim HI, Yamamoto J, Han Q, Sun YU, Nishino H, Tashiro Y, Sugisawa N, Tan Y, Choi HJ, Nam SJ, Bouvet M, Hoffman RM: Response of triple-negative breast cancer liver metastasis to oral recombinant methioninase in a patient-derived orthotopic xenograft (PDOX) model. In Vivo 34(6): 3163-3169, 2020. DOI: 10.21873/invivo.12151

21 Lim HI, Hamada K, Yamamoto J, Han Q, Tan Y, Choi HJ, Nam SJ, Bouvet M, Hoffman RM: Oral methioninase inhibits recurrence in a PDOX mouse model of aggressive triple-negative breast cancer. In Vivo 34(5): 2281-2286, 2020. DOI: 10.21873/invivo.12039

22 Lim HI, Sun YU, Han Q, Yamamoto J, Hoffman RM: Efficacy of oral recombinant methioninase and eribulin on a PDOX model of triple-negative breast cancer (TNBC) liver metastasis. In Vivo 35(5): 2531-2534, 2021. DOI: 10.21873/invivo.12534

23 Kubota Y, Han Q, Hamada K, Aoki Y, Masaki N, Obara K, Baranov A, Bouvet M, Tsunoda T, Hoffman RM: Oral installation of recombinant methioninase-producing *Escherichia coli* into the microbiome inhibits colon-cancer growth in a syngeneic mouse model. Cancer Genomics Proteomics 19(6): 683-691, 2022. DOI: 10.21873/cgp.20351

24 Biglia N, Maggiorotto F, Liberale V, Bounous V, Sgro L, Pecchio S, D'Alonzo M, Ponzone R: Clinical-pathologic features, long term-outcome and surgical treatment in a large series of patients with invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). Eur J Surg Oncol 39(5): 455-460, 2013. DOI: 10.1016/j.ejso.2013.02.007

25 Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MCU, Nielsen TO, Moorman PG, Earp HS, Millikan RC: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. J Am Med Assoc 295(21): 2492, 2006. DOI: 10.1001/jama.295.21.2492

26 Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA: Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 13(15): 4429-4434, 2007. DOI: 10.1158/1078-0432.CCR-06-3045

27 Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im S, Shaw Wright G, Henschel V, Molinero L, Chui SY, Funke R, Husain A, Winer EP, Loi S, Emens LA: Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 379(22): 2108-2121, 2018. DOI: 10.1056/nejmoa1809615

28 Cortes J, Rugo HS, Cescon DW, Im S, Yusof MM, Gallardo C, Lipatov O, Barrios CH, Perez-Garcia J, Iwata H, Masuda N, Torregroza Otero M, Gokmen E, Loi S, Guo Z, Zhou X, Karantza V, Pan W, Schmid P: Pembrolizumab plus chemotherapy in advanced triple-negative breast cancer. N Engl J Med 387(3): 217-226, 2022. DOI: 10.1056/nejmoa2202809

29 Tan Y, Xu M, Tan X, Tan X, Wang X, Saikawa Y, Nagahama T, Sun X, Lenz M, Hoffman RM: Overexpression and large-scale production of recombinantl-methionine-α-deamino-γ-mercaptomethane-lyase for novel anticancer therapy. Protein Expr Purif 9(2): 233-245, 1997. DOI: 10.1006/prep.1996.0700

30 Takakura T, Ito T, Yagi S, Notsu Y, Itakura T, Nakamura T, Inagaki K, Esaki N, Hoffman RM, Takimoto A: High-level expression and bulk crystallization of recombinant l-methionine γ-lyase, an anticancer agent. Appl Microbiol Biotechnol 70(2): 183-192, 2006. DOI: 10.1007/s00253-005-0038-2

31 Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H: Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol 18(7): e3000411, 2020. DOI: 10.1371/journal.pbio.3000411

32 Sun X, Tan Y, Yang Z, Li S, Hoffman RM: A rapid HPLC method for the measurement of ultra-low plasma methionine concentrations applicable to methionine depletion therapy. Anticancer Res 25: 59-62, 2005.

33 Kawaguchi K, Han Q, Li S, Tan Y, Igarashi K, Kiyuna T, Miyake K, Miyake M, Chmielowski B, Nelson SD, Russell TA, Dry SM, Li Y, Singh AS, Eckardt MA, Unno M, Eilber FC, Hoffman RM: Targeting methionine with oral recombinant methioninase (o-rMETase) arrests a patient-derived orthotopic xenograft (PDOX) model of BRAF-V600E mutant melanoma: implications for chronic clinical cancer therapy and prevention. Cell Cycle 17(3): 356-361, 2018. DOI: 10.1080/15384101.2017.1405195

34 Mastrototaro L, Sponder G, Saremi B, Aschenbach JR: Gastrointestinal methionine shuttle: Priority handling of precious goods. IUBMB Life 68(12): 924-934, 2016. DOI: 10.1002/iub.1571

35 Synlogic Initiates Phase 1 Study of SYNB1353 for the Treatment of Homocystinuria (HCU). Available at: https://investor.synlogictx.com/news-releases/news-release-details/synlogic-initiates-phase-1-study-synb1353-treatment [Last accessed on September 12, 2023]

36 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, 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. DOI: 10.3389/fonc.2022.1009548

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

38 Aoki Y, Tome Y, Han Q, Yamamoto J, Hamada K, Masaki N, Kubota Y, Bouvet M, Nishida K, Hoffman RM: Deletion of MTAP highly sensitizes osteosarcoma cells to methionine restriction with recombinant methioninase. Cancer Genomics Proteomics 19(3): 299-304, 2022. DOI: 10.21873/cgp.20321

39 Yamamoto J, Aoki Y, Inubushi S, Han Q, Hamada K, Tashiro Y, Miyake K, Matsuyama R, Bouvet M, Clarke SG, Endo I, 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. DOI: 10.21873/cgp.20299

40 Aoki Y, Tome Y, Han Q, Yamamoto J, Hamada K, Masaki N, Bouvet M, Nishida K, Hoffman RM: Histone H3 lysine-

trimethylation markers are decreased by recombinant methioninase and increased by methotrexate at concentrations which inhibit methionine-addicted osteosarcoma cell proliferation. Biochem Biophys Rep 28: 101177, 2021. DOI: 10.1016/j.bbrep.2021.101177

41 Aoki Y, Yamamoto J, Tome Y, Hamada K, Masaki N, Inubushi S, Tashiro Y, Bouvet M, Endo I, Nishida K, Hoffman RM: Over-methylation of histone H3 lysines is a common molecular change among the three major types of soft-tissue sarcoma in patient-derived xenograft (PDX) mouse models. Cancer Genomics Proteomics 18(6): 715-721, 2021. DOI: 10.21873/cgp.20292

42 Yamamoto J, Aoki Y, Han Q, Sugisawa N, Sun YU, Hamada K, Nishino H, Inubushi S, Miyake K, Matsuyama R, Bouvet M, Endo I, Hoffman RM: Reversion from methionine addiction to methionine independence results in loss of tumorigenic potential of highly-malignant lung-cancer cells. Anticancer Res 41(2): 641-643, 2021. DOI: 10.21873/anticanres.14815

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