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Lowering and Stabilizing PSA Levels in Advanced-prostate Cancer Patients With Oral Methioninase

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Abstract. *Background/Aim: Methionine addiction is a general and fundamental hallmark of cancer due to the excess use of methionine for transmethylation reactions, termed the "Hoffman Effect". Methionine addiction has been shown to be a highly-effective target for cancer therapy by methionine restriction with oral recombinant methioninase (o-rMETase) in preclinical studies, including patient- derived orthotopic xenograft (PDOX) mouse models of cancer. A clinical study of o-rMETase as a supplement showed a 70% reduction of PSA levels in a patient with bone-metastatic prostate cancer. Materials and Methods: In the present study, two advanced prostate-cancer patients took o-rMETase as a supplement for approximately one month. Results: One of the patients taking o-rMETase showed a 38% reduction of PSA levels and the second patient showed a 20% PSA reduction. Conclusion: o-rMETase shows promise for treating patients with advanced prostate cancer.*

Methionine addiction is a general and fundamental hallmark of cancer that greatly distinguishes all tested cancer types from normal cells, with respect to their high requirement levels for methionine (1-8). The methionine addiction of cancer cells is due to excess transmethylation reactions, compared to normal cells, which leads to methionine overuse and depletion of the cellular pools of free methionine and S-adenosylmethionine (7, 8), which is called the "Hoffman Effect" (9-11), a possibly stronger effect than the Warburg effect for glucose (12). The elevated transmethylation in cancer is due in part to the over-methylation of histone H-3 lysine marks (13, 14).

Targeting methionine addiction in cancer with recombinant methioninase (rMETase), cloned from *P. putida* and expressed in *Escherichia coli* (15), has been shown to be effective against all major cancer types in mouse models of cancer, including patient-derived orthotopic xenograft (PDOX) mouse models (16-42).

Previous preliminary safety studies on *i.v.* PEGylated r-METase in macaque monkeys showed that rMETase depleted plasma methionine to <5 μmol/l, with transient anemia being the only side effect (43). PEGylation of rMETase was necessary to prevent anaphylaxis due to repeated treatment (43). *i.v.* injection of rMETase also caused rapid loss of the rMETase co-factor pyrodoxal 5-phosphate (PLP) and thereby, a loss of its enzymatic activity (43).

A previous pilot Phase I clinical trial of o-rMETase was carried out to determine rMETase toxicity and the extent of methionine depletion in high-stage cancer patients. Circulating methionine levels were lowered to 0.1 μM by short-term *i.v.* rMETase treatment, without toxicity (44, 45).

The discovery that rMETase could be effectively administered orally (o-rMETase) (26), solved the problem of anaphylaxis and dissociation of PLP from rMETase, since o-rMETase exerts its effects in the gastro-intestinal tract without entering the circulation. o-rMETase has been shown to be highly effective against recalcitrant sarcoma, pancreatic cancer, colon cancer, and melanoma in PDOX mouse models (17-20, 24-26, 32, 33, 39).

Prostate cancer is the second-leading cause of death of North American men (46). Prostate-specific antigen (PSA) is a blood biomarker of prostate cancer and its progression (46). Androgen-deprivation therapy has been combined with docetaxel to improve survival of prostate cancer patients (46-49). However, survival of hormone-independent prostate cancer patients is low (47), and in need of improved therapy.

Due to the very low toxicity of o-rMETase, a pilot clinical study of o-rMETase, as a supplement, was initiated in 2020, with an advanced prostate-cancer patient with bone-metastatic disease. During a three-month period of receiving o-rMETase as a supplement, the patient had a 70% drop in his PSA levels, without any side effects (50). In the present study, we report a reduction in PSA levels in two additional advanced prostate cancer patients taking o-rMETase as a supplement, without side effects.

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Materials and Methods

rMETase production and formulation. rMETase was fermented in recombinant *E. coli* and purified using column chromatography, as previously described (15). Pure methioninase was dissolved in phosphate-buffered saline (PBS) at 5 mg/ml (250 units), which comprised one dose for oral administration, to be taken after breakfast and dinner.

Methionine measurement. Methionine was measured in the plasma by high performance liquid chromatography as previously described (51).

Results

Patients. Two prostate-cancer patients were treated with o-rMETase twice a day with a dose of 250 units each time. Patient #1 was 55 years old, and had a 35 mm prostate cancer at diagnosis. The patient was initially treated with image-guided radiotherapy. This patient had a Gleason score of 4+3, with perineural invasion and extracapsular extension. Patient #2 was 90 years old, and was diagnosed with prostate cancer, with rapidly rising PSA levels.

PSA reduction by o-rMETase. Patient #1 had a 38.3% PSA drop within four weeks (Figure 1). Patient #2 had a 20.1% PSA drop within four weeks which then stabilized (Figure 2).

Circulating methionine reduction by o-rMETase. Patient #1 had a 42.7% drop in methionine levels during a 12-day period of taking o-rMETase (Figure 3).

Discussion

o-rMETase has been previously shown to cause a 70% drop in the PSA levels of a bone-metastatic prostate-cancer patient who was treated with o-rMETase as a supplement for 3 months, with a starting PSA value of over 2000 ng/ml (50).

Initially, rMETase was administered intra-venously or intra-peritoneally in mouse models (1, 21, 44, 45). However, primate studies showed that repeated *i.v.* infusion of rMETase caused anaphylaxis, which could be prevented by PEGylation of rMETase (43). In 2018, it was found that rMETase could be effectively administered orally (o-rMETase) (26), and subsequent studies showed that o-rMETase was effective in PDOX models against all major cancer types (17-20, 24-26, 32, 33, 39). o-rMETase does not appear to enter the blood stream, thereby eliminating the risk of anaphylaxis, as well as eliminating loss of activity due to dissociation of the rMETase co-factor PLP. o-rMETase functions in the gut to degrade methionine, which subsequently lowers the blood levels of methionine, since there is no longer a source of external methionine (26).

o-rMETase is therefore much safer than injecting rMETase into the blood stream (52), and has allowed the initial development of o-rMETase as a supplement for patients. The fact that the origin of o-rMETase was in *P. putida*, a soil bacterium that survives in environments with a wide range of pH, enabled o-rMETase to evolve into an acid-resistant enzyme that can survive the low pH of the stomach (1, 26).

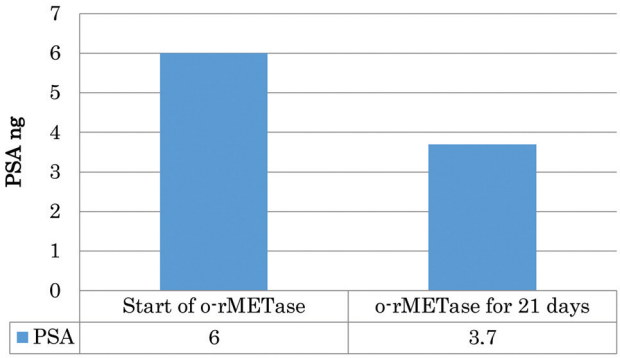

Figure 1. *PSA levels in patient #1 before and after receiving o-rMETase as a supplement for 21 days. The patient took two daily doses of 250 units of o-rMETase.*

The two prostate-cancer patients in the present report responded to o-rMETase as observed by the reduction in PSA levels (Figures 1 and 2). Patient #1 periodically fasted and was on a vegan diet, which may have affected his methionine and PSA levels.

The present dose of 250 units twice a day may be modified in the future in order to maximally lower the circulating methionine levels. Modifying the diet with low methionine–containing foods (53), may also be helpful along with altered dosing and scheduling of o-rMETase, to lower methionine and PSA levels even further. After optimization of the dose, schedule and diet, cancer patients can be treated with combinations of o-rMETase and chemotherapy, as methionine restriction and chemotherapy were found to be synergistic (6, 36). Most promising is combination chemotherapy along with o-rMETase for blockade of the methionine-methylation axis (54-56).

Conflicts of Interest

The Authors report no conflicts of interest in relation to this study.

Authors' Contributions

Qinghong Han produced the recombinant methioninase and analyzed the data. Robert M. Hoffman wrote the paper.

Acknowledgements

This paper is dedicated to the memory of John Mays, AR Moossa, MD, Sun Lee, MD, Professor Li Jiaxi and Masaki Kitajima, MD.

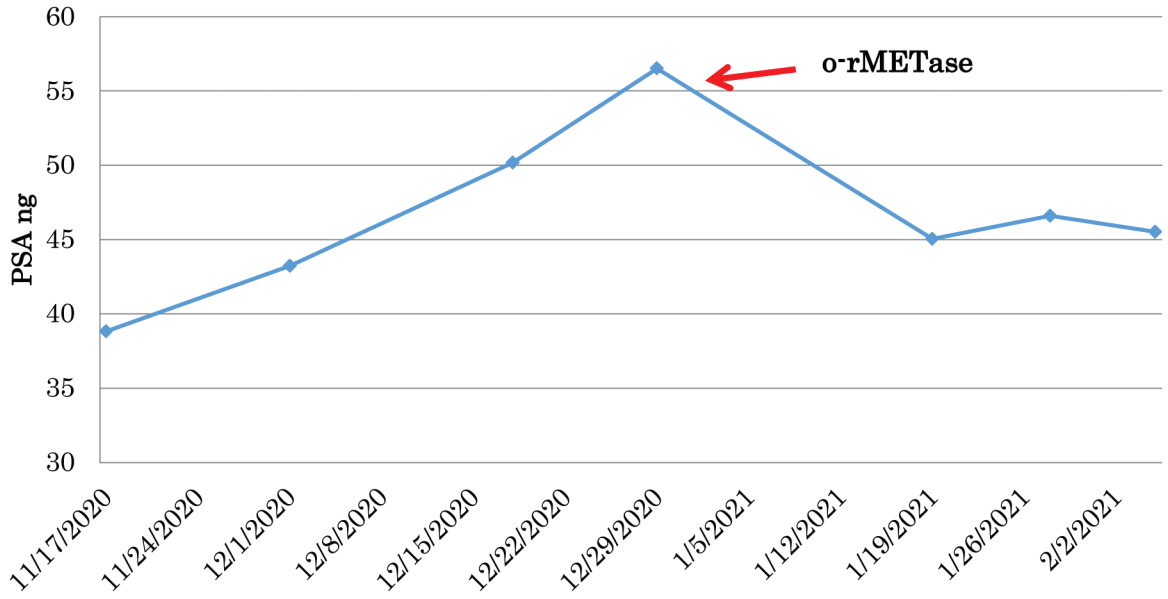

Figure 2. *PSA levels in patient #2 before and during his treatment with o-rMETase, twice daily, 250 units/dose. Red arrow indicates the start of o-rMETase treatment.*

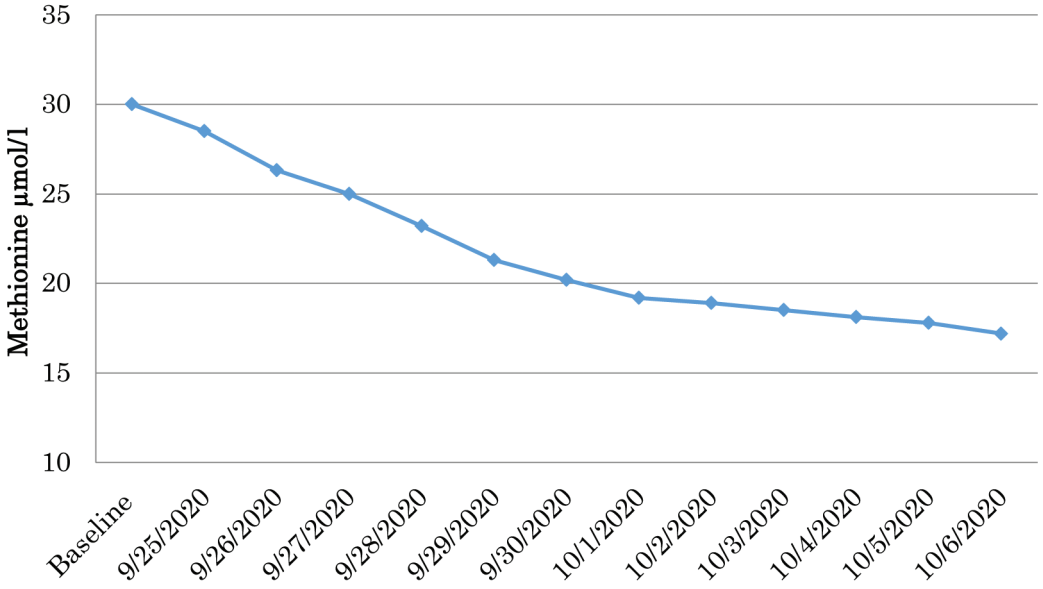

Figure 3. *Daily methionine levels in the plasma of patient #1 during treatment with o-rMETase, twice daily, 250 units/dose.*

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