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Chronic Treatment of an Advanced Prostate-cancer Patient With Oral Methioninase Resulted in Long-term Stabilization of Rapidly Rising PSA Levels

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Abstract. *Background/Aim: Advanced prostate cancer is a recalcitrant disease with very limited treatment options. Our laboratory discovered methionine addiction, presumably a characteristic of all cancer types, including prostate cancer, which can be targeted by methionine restriction (MR), through treatment with oral recombinant methioninase (o-rMETase). Patients and Methods: o-rMETase was produced by fermentation of recombinant E. coli containing the Pseudomonas putida methioninase gene, and purified by column chromatography. An advanced prostate cancer patient received o-rMETase as a supplement, 500 units per day, divided into two oral doses of 250 units each. Results: Before treatment, the patient had a rapid rise in PSA levels, from 39 to 56 ng/ml, within 6 weeks. At the 15th week of o-rMETase administration, the PSA levels stabilized at 62 ng/ml. No overt side effects were observed. Conclusion: o-rMETase single treatment can be beneficial for advanced prostate cancer patients.*

Prostate cancer has extremely limited treatment options and remains a leading cause of death of males in developed countries (1-3). In 1941, Dr. Huggins discovered that surgical castration of prostate cancer patients could slow the disease (4). In the 1970's, Dr. Shally found that a gonadotropin–releasing hormone analog could substitute for surgical castration to control prostate cancer (5). Both, Dr. Huggins and Dr. Schally, won Nobel Prizes for their studies. The problem is that prostate cancer very often becomes resistant to androgen-deprivation therapy (ADT), eventually killing the patient. It was not until 2015 that the chemotherapeutic drug docetaxel was added to ADT, which extended survival of advanced prostate cancer patients (1-3). However, docetaxel displays serious toxicity (1-3).

Methionine addiction is a general and fundamental hallmark of cancer. Methionine addiction is due to much higher requirements for methionine by cancer cells compared to normal cells (6-13). The methionine addiction of cancer cells is due to excess transmethylation reactions in cancer cells. Methionine addiction depletes the cellular pools from free methionine, and S-adenosylmethionine when cancer cells are put under methionine restriction (12, 13). Methionine addiction of cancer is known as the "Hoffman Effect" (14-16). The Hoffman effect is stronger than the Warburg effect for glucose as seen on PET imaging in the clinic (17). The elevated transmethylation in cancer is due in part to the over-methylation of histone H-3 lysine marks, which can at least partially explain methionine addiction (18, 19).

Methionine addiction can be targeted with recombinant methioninase (rMETase). The rMETase gene was cloned from *P. putida*, expressed in Escherichia coli (6, 20), and found to be effective against all major cancer types, as shown for example in patient-derived orthotopic xenograft (PDOX) mouse models (21-44).

Intravenous (iv) injection of PEGylated rMETase depleted plasma methionine to <5 μmol/l, with transient anemia being the only side effect in macaque monkeys; iv-injected PEGylated rMETase did not cause anaphylaxis after repeated treatment (45). In contract repeated *iv* injection of non-PEGylated methioninase caused anaphylaxis (45). The rMETase co-factor pyrodoxal 5-phosphate (PLP) was rapidly lost after *iv* injection resulting in loss of enzymatic activity (45). A pilot Phase I clinical trial of *iv*-rMETase showed rapid methionine depletion to 0.1 μM in advanced-stage cancer patients (46, 47).

Successful oral administration of rMETase (o-rMETase) in mouse models of cancer (21-26, 29, 31, 38, 44), overcame the problem of anaphylaxis and the dis-association of PLP from rMETase. o-rMETase has been shown to be highly

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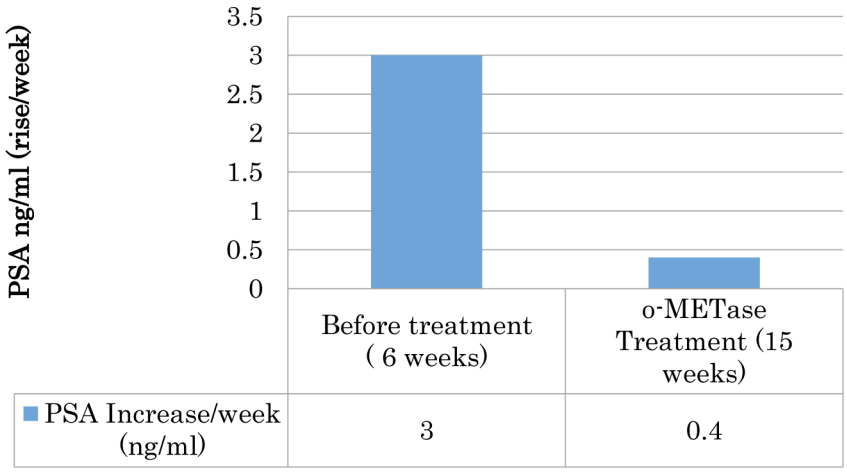

Figure 1. *Rate of increase in the PSA levels of an advanced prostate-cancer patient before and after o-rMETase treatment. o-rMETase was administered at a dose of 5 mg/250 units, twice daily.*

effective against sarcoma, pancreatic cancer, colon cancer, and melanoma in patient-derived orthotopic xenograft (PDOX) mouse models (21-26, 29, 31, 38, 44). Recently, o-rMETase was tested as a supplement in advanced prostate cancer patients and shown to both stabilize and lower PSA levels (48, 49).

Patients and Methods

o-rMETase was produced by fermentation of recombinant *E. coli* containing the *Pseudomonas putida* methioninase gene, and was purified by column chromatography. An advanced prostate cancer patient was administered o-rMETase as a supplement, 500 units per day, divided into two oral doses of 250 units each, after breakfast and after dinner. PSA was measured by standard protocols (6, 20).

Results and Discussion

During the six weeks prior to starting the o-rMETase treatment, the patient's PSA levels increased 3 ng/ml each week from 38 to 56 ng/ml. Following 15 weeks of o-rMETase treatment the patient's PSA levels increased by only 0.4 ng/ml per week (Figure 1).

The treatment for prostate cancer has not been substantially improved since the discoveries of Huggins (4) and Schally (5) on the hormonal control of the disease, which is called androgen-depletion therapy (ADT) (1-3). Although docetaxel has shown survival benefit when combined with ADT, it is associated with significant toxicity (1-3). The present report and our two previous reports (48, 49) indicate that methionine-deprivation therapy (MDT) is a potential new therapeutic approach for prostate cancer.

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 2,000 ng/ml (48). o-rMETase has been shown to lower and stabilize PSA levels in 2 other advanced prostate cancer patients (49).

o-rMETase is much safer than *iv* injected rMETase (45). o-rMETase is being initially developed as a supplement for cancer patients. o-rMETase originated in *P. putida*, where it can survive and proliferate in a wide range of pH and temperatures. Therefore, o-rMETase could have been evolved into an enzyme that can survive in the low pH of the stomach (31) which is a great advantage over a human engineered methioninase which must be injected (50).

Currently, cancer patients are administered 5 mg (250 units) of o-rMETase twice per day (48, 49). The dose and schedule will be optimized in the future, depending on clinical results. It is possible that a low-methionine diet will enhance the efficacy of o-rMETase for prostate and other cancers (51). Since methionine restriction is synergistic with chemotherapy (11, 52-54), o-rMETase can be combined with chemotherapy in future studies. A promising combination is that of o-rMETase, an inhibitor of S-adenosylmethionine synthase, and an inhibitor of DNA methylation, which can block the methionine-methylation axis (41, 55).

We have also shown that the addition of docetaxel or paclitaxel to o-rMETase is beneficial (56, 57) and that patients on ADT and docetaxel may also benefit from the addition of a taxane to o-rMETase.

Conflicts of Interest

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

Authors' Contributions

Qinghong Han produced and purified recombinant methioninase; Robert M. Hoffman and Qinghong Han wrote and revised the manuscript.

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