

## **Changes in the rate of PSA progression and the level of alkaline phosphatase during high dose vitamin C treatment of patients with prostate cancer**

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### **ABSTRACT**

**Introduction:** Intravenously administered vitamin C (IVC) may have anti-cancer and anti-inflammatory properties. Many studies demonstrated evidence of a good safety profile of IVC treatments and improvement of the quality of life in cancer patients. IVC has been proposed as a treatment for cancer as an adjuvant in conjunction with other therapies. To investigate high dose ascorbic acid potential in treating prostate cancer, a retrospective study was conducted using clinical data from the Riordan Clinic database (1994-2015).

**Methods:** We collected data, when available, on the following patient characteristics at diagnosis and during the courses of IVC therapy: age, tumor stage, Gleason score, serum prostate specific antigen (PSA) and alkaline phosphatase (ALP) levels, and location of metastases. In particular, PSA, ALP, and C-reactive protein (CRP) levels are analyzed in prostate cancer patients given IVC therapy during several years.

**Results:** We found that PSA, CRP, and ALP correlate with tumor staging as measured by Gleason scores. Moreover, peak plasma ascorbate levels attained during the patients first IVC infusions are reduced in patients with elevated PSA and CRP levels. Tracking the changes in PSA and ALP with time in patients for whom data are available indicates that the rate of increase in these variables over time can be reduced by incorporating IVC therapy and by increasing the frequency of IVC treatments.

**Conclusion:** There appeared to be a relation between the frequency of IVC treatments and the rate of PSA change, with PSA rate of growth decreasing as the frequency of IVC increases. Further research into the use of IVC in prostate cancer patients is warranted.

**Key words:** High dose vitamin C, prostate cancer, prostate specific antigen, alkaline phosphatase, C-reactive protein.

## INTRODUCTION

Vitamin C (ascorbic acid, AA) is an essential water-soluble antioxidant that plays significant roles in immune function, mediation of inflammation, and tissue repair via collagen production [1]. It has been studied extensively for its potential in preventing chronic diseases [2].

Intravenous high dose vitamin C (IVC) is a commonly used therapy among naturopathic doctors and other integrative oncology healthcare practitioners. Many studies demonstrated evidence of a good safety profile and potentially important anti-tumor activity of high-dose vitamin C [3-6]. IVC treatments have been reported to improve the quality of life in cancer patients [7, 8] and have been proposed as a treatment for cancer as an adjuvant in conjunction with other therapies [9-11].

There are several potential motivations for using IVC in cancer therapy. For instance, cancer patients are often deficient in vitamin C, and this deficiency is correlated with decreased dietary intake, increased inflammation, and reduced survival time [12].

The anticancer mechanisms of vitamin C are not fully understood. Several mechanisms were proposed such as the effects of high doses of vitamin C on angiogenesis, suppression of inflammation, modulation of gene expression, and improvement of immune cells' functioning [13-16]. There are in vitro and animal studies that showed that high-dose vitamin C induces pro-oxidant effects and selectively kills cancer cells [17, 18]. Increased reliance of cancer cells on glycolysis suggested that following IVC treatment elevated uptake of oxidized ascorbic acid may cause oxidative stress by depleting glutathione [19], as well as the mechanism of extracellular delivering of hydrogen peroxide to tumor tissue.

As ascorbate has been shown to stimulate the production of type I and III collagen [20], the stimulation of collagens' production in the extracellular matrix can be important components of the physical barrier against invasion and metastasis of cancer cells.

Recent studies indicate that vitamin C may have important effects on gene expression. Since the induction of HIFs may be mediated by ROS, antioxidants such as vitamin C may inhibit HIF-1 and HIF-1a expression; this is supported by experimental studies [21]. Vitamin C may also act on HIF-1 expression by inhibiting the expression of Nuclear Factor kB (NF-kB) genes. NF-kB is involved in inflammation and tumor development [22]. Ascorbate has been shown to inhibit the expression of NF-kB [23, 24]. The anticancer effect of ascorbate may be in part due to its ability to suppress specificity protein (SP) transcription factors [25]. Sp-regulated genes are involved in cancer proliferation, survival, and angiogenesis. Ascorbate was also shown to suppress tumor growth through the modulation of p53 [26]. This creates a rationale for using high dose ascorbate as an anti-tumor agent.

Oxidative stress and inflammation are key developments in prostate cancer progression, and the strong correlation between C-reactive protein, an inflammation marker, and prostate specific

antigen, a cancer marker, suggests that chronic inflammation may be a legitimate target in prostate cancer treatment [27]. Inflammation is fundamental in prostate cancer: evidence implicates it in prostate cancer development and progression to advanced metastatic disease [28]. In many cancers, inflammatory gene markers are correlated with poor prognosis [29].

IVC therapy reduced CRP levels and decreased plasma concentrations of several inflammatory and angiogenic cytokines; moreover, these decreases correlated with reductions in the levels of tumor markers [15, 30]. Prostate cancer may be a particularly promising candidate for IVC therapy. Several studies show prostate tumor cells in vitro, in addition to implanted prostate tumors in animals, are sensitive to ascorbate [31-34]. Moreover, a clinical study combining vitamin C and vitamin K<sub>3</sub> in prostate cancer patients who failed standard therapy showed promise in delaying biochemical progression, as measured by decreases in PSA velocity and doubling time [35].

Since the Riordan Clinic has been treating cancer patients with IVC for decades, we have a potentially useful database of patients with prostate cancer and IVC. In the present manuscript, we examined our historic data for prostate cancer patients given IVC, with a particular focus on tracking changes in PSA levels and—where data are available—alkaline phosphatase levels. PSA is a reliable, sensitive, easy to measure, and widely used biomarker for prostate cancer. There is some indication that declines in PSA levels are predictive of longer patient survival [36-38]. ALP is particularly useful as an indicator of osteoblastic activity [39], and may in fact be a better predictor of survival than PSA [40].

## **METHODS**

We analyzed the data of prostate cancer patients from the Riordan Clinic (Wichita, KS) who were treated with IVC and who had PSA and ALP levels measured at some point before and after treatment.

The study was conducted under Institutional Review Board Approval of Riordan Clinic. Demographics were limited to ensure confidentiality.

Using the LabNet laboratory management program (Henry Schein, Melville, NJ), we collected data from 180 prostate cancer patients when available, on the following patient characteristics at diagnosis and during the courses of IVC therapy: age, tumor stage and grade at diagnosis, Gleason score, serum PSA and ALP levels, location of metastases (if present), and survival time (time from diagnosis to death). Note that not all of these parameters were available in the database for all patients: for example, Gleason scores were only available for roughly half of the subjects, and many of the patients were lost to follow up. Averages for available values are given in Table 1, along with the percentages of patients who had conventional treatments and the survival times.

Most of the patients were treated by IVC after conventional treatment: surgery, chemotherapy, or radiation. 16 patients refused conventional treatment and choose alternative treatment. For all patients treated by IVC the periods of analysis were chosen when patients did not have conventional treatment.

Obviously, in a retrospective study, such as one covering 20 years of research, protocols and doses varied from subject to subject. However, in general IVC was administered in accordance with the Riordan IVC protocol [5]. Briefly, new cancer patients were given a 15-gram ascorbate infusion for their first dose, followed by a 25 gram infusion on day two. Dosage was then gradually

adjusted upward by the physician, based on patient tolerance for the treatment and the plasma ascorbate levels attained, to maximum doses between 50 grams and 100 grams. Frequencies of treatments ranged from several times per week to once-per-month.

**Table 1.** Parameters for patients used in retrospective study of IVC therapy for prostate cancer

Parameters for patients used in retrospective study of IVC therapy for prostate cancer			
Number of Patients	180		
Tumor Stage Breakdown	31 (T2), 6 (T3), 5 (T4)		
Gleason Score Breakdown	25 ( $\leq 7$ ); 45 ( $>7$ )		
Conventional Treatment	Yes (80); No (16)		
Metastasis (Bone Scan)	22		
Age (years)			
Start of Treatment	66.6 $\pm$ 8.5 (mean $\pm$ SD)		
Time of Death	75.7 $\pm$ 9.2 (mean $\pm$ SD)		
Survival Time	7.6 $\pm$ 5.6 (mean $\pm$ SD)		
Serum PSA (ng/mL)			
Initial	7.7 (Median)	2.9 – 26.0 (IQR)	98.1 (Mean)
Maximum	12.3 (Median)	4.5 – 53.0 (IQR)	846 (Mean)
ALP (IU/L)	123.5 (Median)	40 – 3118 (IQR)	

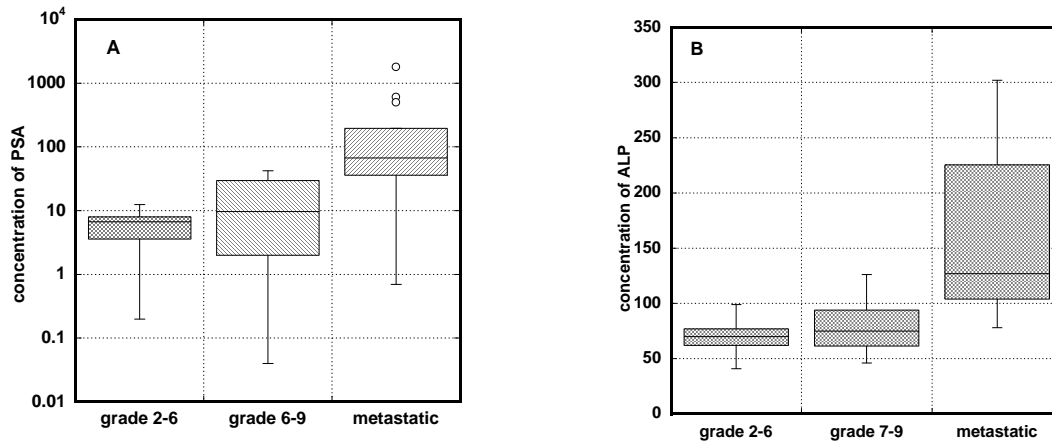
All laboratory tests were conducted by the Riordan Clinic Bio-Center Laboratory, a licensed and certified medical laboratory working in full compliance with HIPPA regulations and using standard methodologies. Plasma ascorbate concentrations were measured by HPLC with electrochemical detection. Serum PSA concentrations were determined using an equimolar immunoassay (PSA kit, Abbott Laboratories, UK). According to this protocol, the upper normal range for PSA is 4.0 ng/mL. Serum ALP was measured using a standard commercially available assay, with the normal range in our laboratory being 30U/L-110 U/L.

Statistical analyses were carried out using the Excel spreadsheet program, while graphs with the least squares regression data fits where appropriate were constructed using the Kalaidagraph program (Synergy Software, Reading PA).

**RESULTS**

Our database contained a total of 180 prostate cancer patients. However, complete information on the tumor stage, presence or absence of metastases, and history concerning the use of conventional therapies were not available for many of these subjects. The effect of the tumor stage on the values of two key cancer markers PSA and ALP is shown in Figure 1. Increases in these markers with

clinical stage are shown here, in addition to that of metastases, and are as expected based on prior reports.

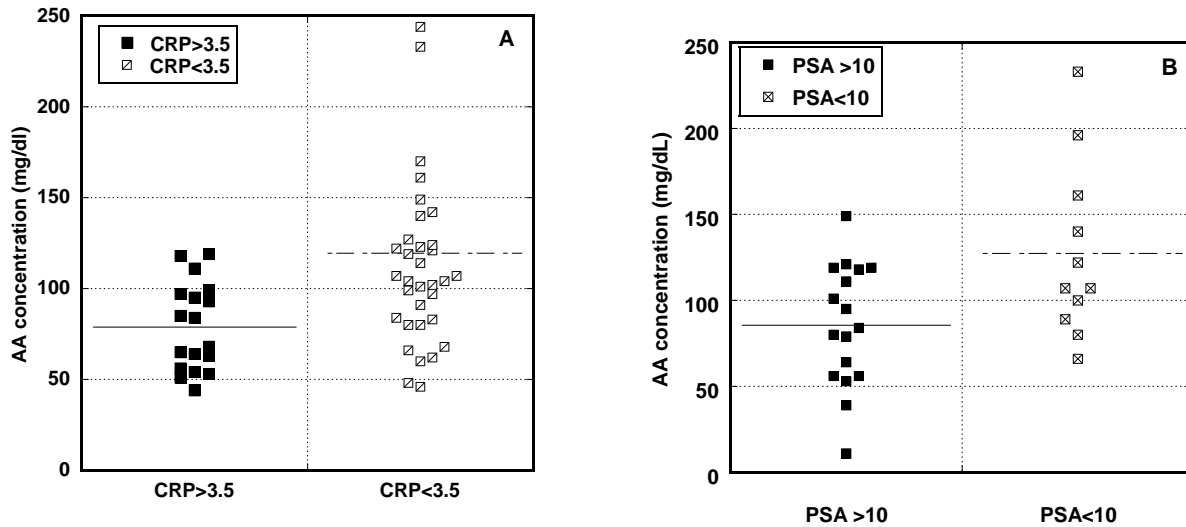


**Figures 1 (A, B).** Relationships between tumor stage and serum concentrations of PSA (A) and ALP (B). The stages of prostate cancer were divided by Gleason scores of 6 or less, without metastases; Gleason scores of 7 or more, without metastases; Gleason Scores of 7 or more, with metastases. Box plots indicate quartile ranges (1st quartile to 3rd quartile in box, with line inside box representing a median value).

The distributions of PSA for different states of diseases are shown in Figure 1A. PSA increased with an increasing clinical stage, and patients with metastasis had a statistically higher level of PSA than patients with localized tumor ( $P < 0.001$ ). Distributions of ALP for different Gleason scores and localized or metastatic diseases are shown in Figure 1B. According to these data the significant increase in ALP was measured for metastatic state of the cancer ( $p < 0.03$ ). ALP averages were  $70 \pm 14$  U/L for grades 2-7,  $79 \pm 24$  U/L for grades 7-9 and  $196 \pm 127$  U/L for metastatic stage of disease.

For many subjects, ascorbate pharmacokinetic data were available. Concentration of ascorbic acid was measured in plasma before and after IVC. High doses of injected ascorbic acid intravenously produced the average peak plasma AA concentrations ranged from 5 mM (88 mg/dL) at dosage 15 grams to the levels 10 mM (176 mg/dL) and 14 mM (246 mg/dL) at dosages 25 grams and 50 grams [5]. Typically with IVC, the elimination half-life at such dosages is roughly two hours [41].

Initial IVC treatments (15 gram dose, infused over a one-hour period) led to peak plasma ascorbate concentrations in cancer patients that varied considerably from patient to patient. As shown in Figure 2, patients who had lower PSA levels, or lower CRP levels, attained higher peak plasma ascorbate concentrations



**Figures 2 (A, B).** Relationships between CRP (A) or PSA (B) concentrations and the peak plasma ascorbate concentration attained in the patient’s first IVC infusion. PSA and CRP concentrations separated to less than 10 ng/mL for PSA or less than 3.5 mg/L for CRP or above those thresholds ( $p < 0.002$  for CRP and  $p < 0.03$  for PSA). Lines represent median values.

These differences were statistically significant ( $p < 0.002$  for CRP and  $p < 0.030$  for PSA), and are consistent with earlier studies indicating that cancer patients have lower peak ascorbate levels compared to healthy adults, and the achievable levels of AA in plasma decreases with increased levels of inflammation and the progression of disease [42].

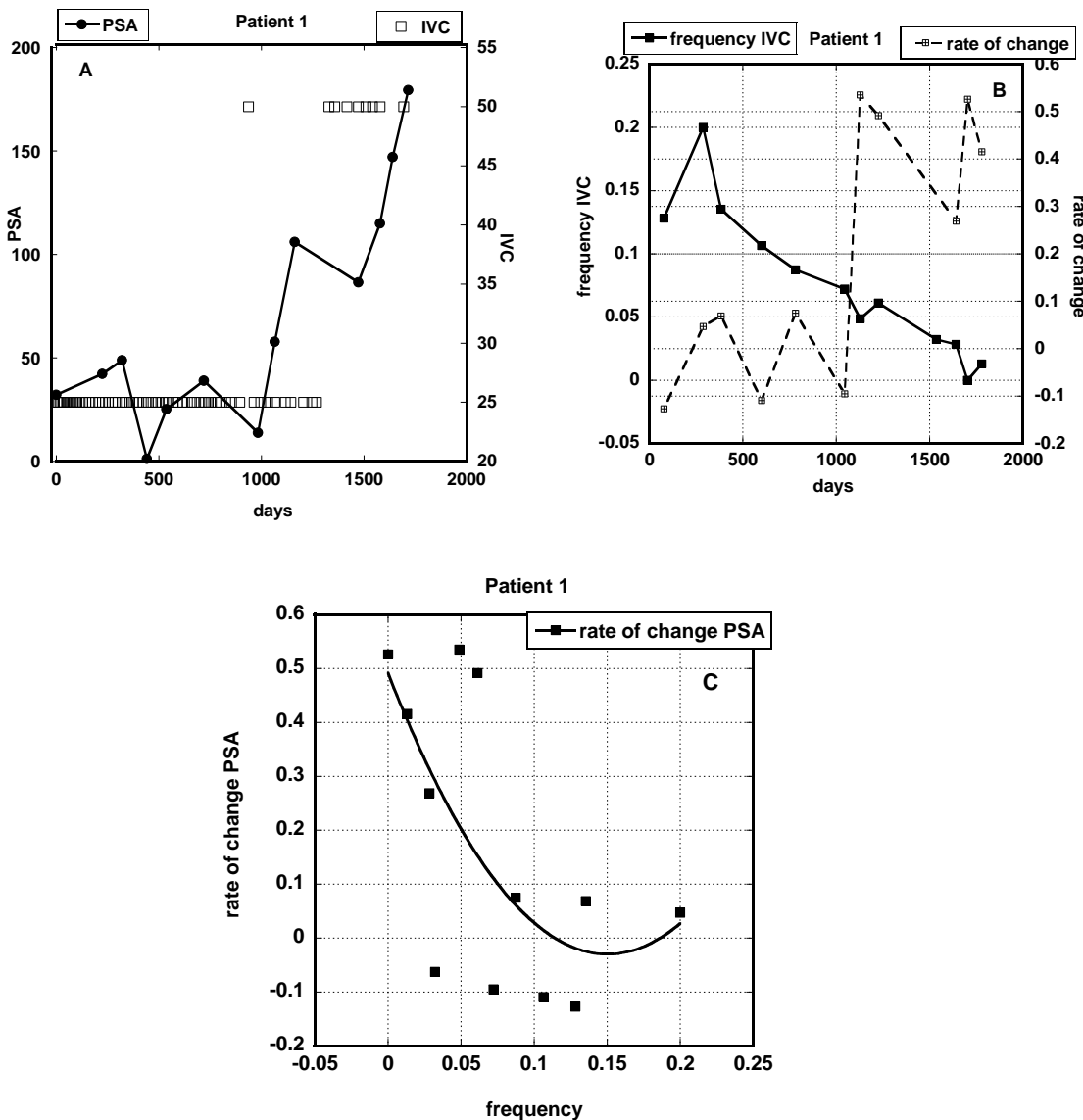
Based on the idea that conditions like cancer and chronic inflammation lead to lower ascorbate levels and higher levels of oxidative stress, it makes sense that subjects with a higher tumor burden (as indicated by PSA) or a greater degree of chronic inflammation (as indicated by CRP) would have lower ascorbate levels. Subsequent IVC treatments at the same dose typically yield lower plasma ascorbate concentrations as depleted tissue stores are replenished.

The overall efficacy of IVC against cancer for all prostate cancer patients, as measured by PSA or CRP levels, was difficult to evaluate for a variety of reasons. In some cases, patients discontinued treatments after a relatively short time from the beginning. In others, key lab parameters (such as PSA) were not adequately tracked. Moreover, patients varied considerably in terms of what other treatments (conventional or alternative) they received in addition to IVC.

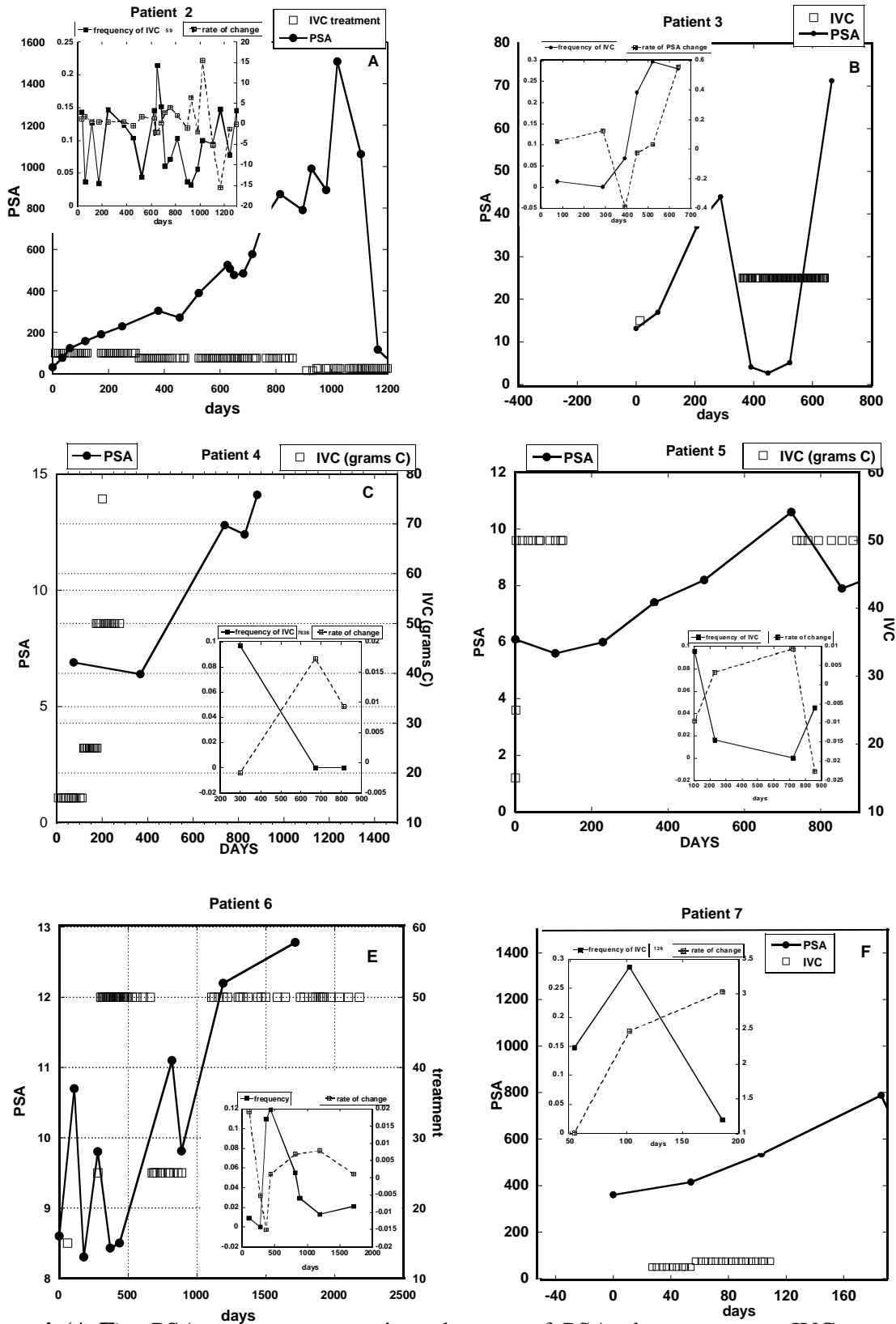
There were some subjects who were treated with IVC for a regular and prolonged period and for whom PSA levels were monitored at regular intervals. In these subjects at the periods when they did not have conventional treatment, we were able to analyze how IVC treatments affected PSA levels and PSA velocity. In doing so, we define the PSA velocity (PSAV), the change in PSA concentration per day between measurements, and the IVC treatment frequency (IVCf), the number of IVC treatments per day between PSA measurements:

- $PSAV = (PSA_2 - PSA_1) \div (t_2 - t_1)$  units of ng/mL/day
- $IVCf = \{\text{Number of IVC treatments over interval } t_1 - t_2\} \div (t_2 - t_1)$  units of day<sup>-1</sup>

An example of how these variables tracked each other in one patient (denoted “Patient 1”) is shown in Figures 3A – 3C. Data in Figure 3 show PSA concentration (ng/mL) versus time along with indications of IVC treatments in grams, the change in PSA concentration, and IVC treatments per day, in addition to correlate] on between PSAV and IVCf. The data points were approximated by regression line:  $y = 0.49 - 6.9 x + 23.1x^2$  ( $r = 0.66$ ).



**Figure 3 (A-C).** Data for Patient 1: (A) PSA concentration (ng/mL) versus time, along with indications of IVC treatments in grams given. (B) The rate of PSA concentration change and frequency of IVC (treatments per day) versus time. (C) Correlation between PSAV and IVC frequency ( $r=0.66$ ).



**Figure 4 (A-F).** PSA concentration, the rate of PSA change, IVC treatments and the frequency of IVC treatments versus time (days) for Patients 2 through 7. Squares on the right Y-axis indicate dosage of IVC treatments and dark circles on left Y-axis—the measured levels of PSA. Values in inserts show the rate of PSA change (right Y-axis) and the frequency of IVC treatment (left Y-axis).



This patient (Patient 1, Figure 3) came to the clinic in 2006. A biopsy performed six months before his visit showed a stage D2 castration resistant tumor with a Gleason score of 8. The patient did not have surgery or hormonal treatments (choosing exclusively homeopathic treatments instead). IVC therapy was first carried out in 2006 and stopped in 2011. In 2012, when the tumor was discovered to be metastatic, the patient began Casodex and Lupron treatments and had surgery (TURP). In 2014, he was placed in clinical trials for Cabozantinib and Taxotere.

Data presented in Figure 3 are related to the period of IVC treatment before chemotherapy and surgery. According to these data for the period about five years, the patient was treated by 150 IVCs with dosages 25 grams and 50 grams with frequencies one or 1.5 times per week, one time per two weeks or at the end of the treatment, one time per month.

It appears from Figure 3A that PSA levels fluctuated without significant increase at first (during the first thousand days) and then began to increase more rapidly. The number of treatments was reduced at this point, as shown in Figure 3B. It can be seen here that as the number of treatments was reduced (and even after the dose per treatment was increased), the PSA concentration rose. The correlation between these two variables is shown in Figure 3C, suggesting that for this patient a reduction in the number of treatments corresponded to higher PSAV values. As a result, the frequency of the treatment the rate of growth of PSA was also changed and increased for less frequent treatments.

Figure 4 shows the tracking of PSA and rate of PSA growth with the frequency of IVC treatments (IVCf) for six other patients (Patients 2 through 7). Inserts in each graph present the same information as in Figure 3B (the dependence with time frequency of IVC on the first Y-axis and rate of PSA change on the second Y-axis). In most of the cases, the decrease in the frequency of the IVC resulted in the increase of the rate of growth of PSA.

A description of each patient is also given:

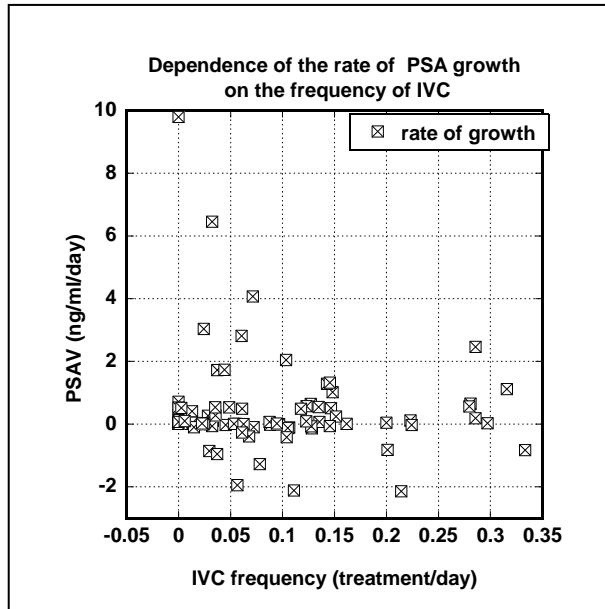
- Patient 2 (Figure 4A) came to the clinic at age 61, his tumor having been evaluated four years earlier with a Gleason score ranging from 6 to 9 (depending on area). The records in the database showed that the patient did not have metastases and did not use conventional therapy. This patient was followed for over seven years, and had more than 230 IVC treatments during this time. For the first three years, he received doses of 75 grams to 100 grams IVC once per week. In the last four years, he was given 25 grams IVC one or two times a week. There were several intervals of roughly a month in duration each during which he received no treatments. The average rate of PSAV changed from  $0.19 \pm 1.27$  ng/mL/day for periods with frequency of injection one per week or higher to  $0.52 \pm 2.15$  ng/mL/day for frequencies of IVC less than one per week. Additionally, for the periods of treatment when the treatment by IVC was even less frequent, the rate of PSAV was increased to  $1.26 \pm 2.61$  ng/mL/day. There were no records of the conventional treatments at the end of the analyzed period, when the PSA level declined.
- Patient 3 (Figure 4B) had transurethral resection of prostate (TURP) five years before he came to the clinic for IVC treatment. He had a Gleason score at diagnosis of 4 and elected nutritional therapies, refusing surgery and radiation. During the next half year he had 42 IVC treatments (25 grams IVC each), with one treatment per 5 days on average. PSAV

decreased from 0.38 ng/ml/day to 0.03 ng/ml/day, and PSA concentration was reduced from 41 ng/mL to 5 ng/mL. During treatment the patient said that he had the best sleep he had in many years. Patient continued treatment for an additional three months and then stopped treatment. One month after the discontinuation of the treatment, he complained about increased pain and his serum PSA increased to 71ng/mL.

- Patient 4 (Figure 4C) was followed for roughly two years. There are no data for this patient concerning tumor stage or the use (or not) of conventional therapy before IVC treatment, probably because of the tumor was early stage. During the first year we followed, he received 29 IVC treatments (with dosages of 15, 25, or 50 grams each). PSA levels were stable during this time between 6 ng/mL to 7 ng/mL. The patient then discontinued treatments. From this point next measurement of PSA after 10 months showed that PSA levels doubled.
- Patient 5 (Figure 4D) came to the clinic in 2007 with a Stage 2 T1cN0M0 tumor that had a Gleason score of 5. He did not have conventional therapy, but instead received IVC treatments. After initial 15 g and 25 g infusions, he received ten treatments of IVC 50 grams each, at a frequency of one or two times per week. During this time period, PSA concentrations remained stable. The patient then discontinued treatment for roughly 1.5 years, experiencing a doubling of PSA concentrations during this time. IVC treatments were then resumed at 50 grams IVC each. This treatment resumption corresponded to a stabilization of the PSA level (a drop in PSA levels from 11 ng/mL to 8 ng/mL over a four-month period).
- Patient 6 (Figure 4E) was diagnosed with prostate cancer at age 68. The tumor stage at the first visit was: Gleason Score 6, T1cN0M0. There is no record of this patient receiving conventional therapy during the five year period during which he had a total of 57 IVC treatments. The analysis of the rate of PSA growth with the frequency of the treatment showed that at the highest frequency of the treatment (once per week) the rate of PSA growth was at level zero and increased at less frequent treatments. This patient's PSA levels generally increased during the five year period over which we have data, but periods of low PSA increase corresponded to higher IVC treatment frequencies.
- Patient 7 (Figure 4F) came to the Riordan Clinic after previously receiving conventional treatment: radiation and two times chemotherapy (Lupron) during four months and one month with three-year interval between treatments. Records indicate that the tumor was not considered malignant at that time, but PSA levels were rising dramatically prior to beginning IVC therapy. The PSA after the last treatment by Lupron decreased to the level 14 ng/ml but then started to increase. The patient came to the clinic and started IVC treatment one and half year after the last conventional treatment when the PSA increased to the level 360 ng/mL. During the analyzed period, patient had 24 IVC with high dosages of 50-75 grams. The rate of PSA growth was suppressed when patient had one IVC per day and increased about three times at the frequency one IVC per month.

The later data showed that after IVC treatment patient was treated by Casodex during one month, the level of PSA stabilized but increased more than 15 times two months after the end of the treatment.

The correlations between the rate growth of PSA and frequency of treatments is shown in Figure 5, with data from a total of fifteen patients for whom detailed PSA concentration vs. time data were available.

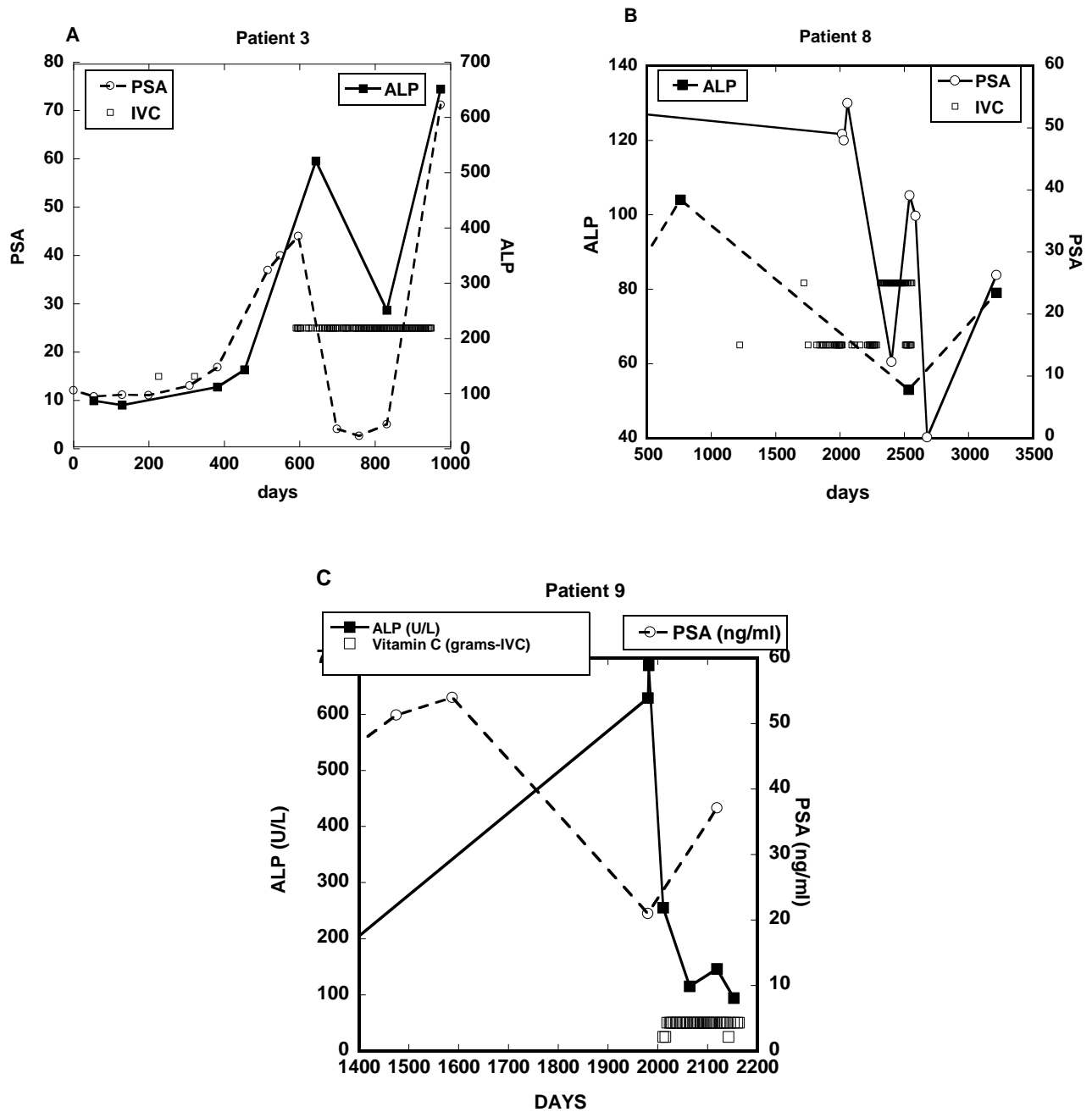


**Figure 5.** Correlation between PSAV (ng/mL/day) and the frequency of IVC treatments (treatments/day) for fifteen prostate cancer patients.

These data show decreases in the rate of PSA concentration growth with increased IVC treatment frequencies. The correlation was not significant at the 0.05 level. For instance, in comparing IVC frequencies of once per week with lower frequencies, the p value was roughly 0.16 and was reduced to 0.06 when values for PSA concentrations above 20 ng/mL were considered. The high variability of the data can be explained by the different stage of the disease of different patients, as the effectiveness of treatment may depend on many factors including the aggressiveness of tumor, initial levels of PSA and inflammation, and the state of immune system. Ultimately, a systematic study is needed, but the data in Figures 3 through 5 suggest that PSA levels are generally less likely to increase when IVC is administered often and sufficiently.

The other parameter we investigated in detail was ALP, a marker of bone formation that can be used to some degree to track bone metastases in prostate cancer patients. Alkaline phosphatase is one of the older biochemical tools for investigating and monitoring prostate cancer, and a reliable indicator of osteoblastic activity and bone metastases.

Since this was not measured nearly as often as PSA, we were confined to examining three subjects who had described ALP vs. time data. The data for these subjects are shown in Figure 6, with descriptions of each patient (Patient 3 described above, in addition to Patients 8 and 9).



**Figure 6 (A-C).** ALP concentration (IU/L) and the PSA concentration (ng/mL) for three cancer patients. Concentrations of PSA are shown by open circles on the right Y-axis and concentrations of ALP are shown by dark squares on the left Y-axis. Open squares indicate times when IVC treatments were given and dosages in grams on Y-axis according to labels.

In these patients, the onset of IVC therapy, and the increasing of its frequency, led to reductions in ALP values. PSA levels also tended to track APL levels for these subjects.

- Patient 3 (Figure 6A) showed ALP increases from 149 U/L to 512 U/L in the six months prior to treatment. After seventy-six IVC treatments at 15 g each, the ALP concentration

decreased to 251 U/L. This also correlated with a decrease in PSA. One month after the discontinuation of IVC therapy, concentrations of PSA and APL showed sharp increases.

- Patient 8 (Figure 6B) came to the clinic in 1994 with diagnosis of prostate cancer. He refused biopsy, bone scan, and conventional therapy. The patient was treated with 15 grams IVC at a frequency of roughly once per week. During the first year of treatment, his PSA concentration decreased from 54 ng/mL to 12 ng/mL, and eventually was reduced to normal levels after another year of treatment. One year without treatment saw his PSA levels increased to 26 ng/mL. ALP levels over time decreased from an initial level of 105 U/L to 53 U/L after 80 IVC treatments and increased to 73 U/L one year after discontinuation of treatment.

Patient 9 (Figure 6C) first came to the Riordan Clinic having a “hard and nodular” prostate. He did not have a biopsy and refused chemotherapy. Seven years later, metastasis was diagnosed in the liver and bone. At this time, the patient’s ALP concentration was 687 U/L. The patient had surgery (cholecystojejunostomy & gastrojejunostomy) prior to starting IVC therapy. Two weeks post-surgery ALP concentration was reduced to 255 U/L. At this point, the patient started 50 gram IVC treatments two times per week. Two months later, the level of ALP decreased to 155 U/L. After 3 months and 24 IVCs ALP decreased further to 94 U/L. During treatment with IVC the patient recorded that he was feeling a lot better. PSA concentrations ranged from 21 ng/mL to 37 ng/mL.

Altogether, these three cases show dynamic changes in alkaline phosphatase during IVC therapy. As ALP suppression is thought to indicate suppression of osteoblast bone formation in prostate cancer patients, the ALP decreases with IVC treatment are encouraged if preliminary.

## DISCUSSION

In an effort to develop effective alternative strategies that increase the therapeutic efficacy and minimize the systemic toxicity of chemotherapeutic agents, many efforts are being directed towards the investigation of nutraceutical agents and in particular high dose vitamin C as a therapeutic agent.

The purpose of this study was to determine if IVC therapy could suppress PSA and ALP levels in prostate cancer patients. Our analysis of the Riordan Clinic cancer database indicates that this may well be the case, and suggests that further systematic clinical studies into this phenomenon are warranted. Our conclusions can be summarized as the following:

1. Consistent with earlier studies, we find that PSA, ALP, and CRP concentrations correlated with Gleason scores and the presence of metastasis in our patient group.
2. In cases where pharmacokinetic data are available, we found that patients with higher Gleason scores and/or with metastases attain lower plasma ascorbate levels with a given dose infusion. This is consistent with earlier reports that cancer patients are deficient in vitamin C and that this deficiency is more pronounced as their disease progresses.

3. There appears to be a correlation between the frequency of IVC treatments and the PSA “velocity,” with PSAV decreasing as IVC frequency increases, although we were not able to demonstrate statistical significance at the 0.05 level.
4. In the few cases where we found both PSA and ALP measurements recorded, these variables tended to track each other, and they tended to both decrease during IVC therapy.
5. In several cases, the reductions in PSA and/or ALP concentrations (or their stabilization) were reversed once the treatment stopped.

As the PSA concentration varies depending upon tumor differentiation, tumor volume, and the extent of disease [37, 38], the relationship between the PSA rate of change and frequencies of IVC treatment may indicate some inhibitory effect of the treatment on the cancer. PSAV, the rate of change in concentration of the PSA marker, is thought to be an important prognostic parameter. Several reports suggest that PSA doubling time can be used to predict the aggressiveness of the disease [43], and may be useful in identifying patients at risk for progression after prostatectomy or radiation therapy.

Our preliminary observations concerning IVC therapy and ALP levels were encouraging. According to a large multivariate analysis of patients with metastatic androgen-independent prostate cancer, elevated serum ALP levels were independently associated with shorter survival [44]. Moreover, ALP levels have been associated with the progression of skeletal metastases in patients with prostate cancer [45] and have also been shown to be significant predictors of early death [46, 47]. While osteoclastic processes are seen as a potential target for prostate cancer therapy, chemotherapeutic drugs aimed at inhibition these processes offer only a few months advantage over placebo in prolonging survival time [48], and often carry side effects such as osteonecrosis of the jaw, hypocalcemia, and deterioration of renal function [49]. IVC therapy is unlikely to have such severe side effects, and may act as an adjuvant to reduce side effects of other agents. Furthermore, ascorbic acid induces formation of collagen matrix and enhances osteoblast differentiation [50]. Since cancer patients are ascorbate deficient and have an abnormal demand for ascorbate due to oxidative stress, using AA as an adjuvant to support these processes may help prevent metastasis in prostate cancer patients.

## **CONCLUSION**

Our study is the first to address dynamic changes of the easily available biomarkers ALP and PSA during alternative therapy by high dose IVC. We underlined the possibility of ALP decline, which is a marker of suppression of osteoblast bone formation marker, suppression of the rate of PSA growth, and the dependence of the suppression of the rate of PSA growth on the frequency of the high dose vitamin C treatment. Both these dependencies resulted from the analysis of the data, demonstrating the clinical benefit of IVC for prostate cancer patients.

This study was ultimately limited by the small number of patients in our database who had sufficiently detailed information about analyzed parameters, frequent PSA, and ALP measurements, in addition to consistent participation in the IVC protocol. A controlled study using a larger population of prostate cancer patients would allow for a more rigorous assessment of the promising trends reported in this study.

**List of abbreviations:** prostate specific antigen (PSA), intravenous vitamin C (IVC), alkaline phosphatase (ALP), C-reactive protein (CRP).

**Competing interests.** The authors declare that they have no competing interests associated with this manuscript.

**Authors' contributions:** Nina Mikirova, PHD, is the principle investigator of this study and contributed to design, data analysis, statistical analysis and writing of the manuscript. Ronald Hunninghake, MD, assisted in interpretation of the results.

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