

Meeting Report

Vitamin C Symposium 2019—“Vitamin C for Cancer and Infection: From Bench to Bedside”

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1. Preface

The Vitamin C for Cancer and Infection symposium was organised in response to recent international clinical trials that have highlighted the potential for vitamin C administration to improve clinical outcomes for patients with severe respiratory illness, sepsis and some cancers. The symposium was held at Auckland University of Technology in New Zealand and we were privileged to have prestigious international clinical researchers from the USA and Europe as presenters, as well as local biomedical researchers and clinicians with expertise in the fields of infection and cancer. Many of these clinicians and researchers presented at the first symposium on vitamin C in the USA in 2017 [1]. The 2019 symposium comprised one day of scientific and clinical sessions, with an overview of vitamin C and mechanisms of action, as well as presentations on the clinical use of vitamin C for cancer and infection. This was an educational event targeted primarily at doctors and nurses and was endorsed by the Royal New Zealand College of General Practitioners (RNZCGP) and registered with the College of Intensive Care Medicine of Australia and New Zealand (CICM) for Continuing Professional Development. The symposium also included a separate afternoon event specifically targeted at the general public with key speakers from the main symposium day. Over 250 people attended the main symposium day and the public event, with much positive feedback.

2. Overview of Vitamin C

2.1. Overview of Vitamin C: New Horizons for Nutrition and in the Clinic

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Vitamin C (ascorbate) is necessary for life in most plants and animals, and is an essential dietary component for humans, primates and a few other species that have lost the capacity to generate this molecule from glucose. Ascorbate is a labile molecule, readily oxidised in the presence of air, and is a well-known reducing agent. It is these properties that underpin its many biological functions. As an antioxidant, ascorbate can react with, and protect the body from, damaging radicals generated by solar radiation and reactive oxidants. In addition, ascorbate can reduce and chelate transition metals, and this property underpins its capacity to act as a co-factor for metal-containing enzymes throughout the body. These enzymes include those that regulate the body's response to hypoxia, that synthesise adrenaline and carnitine, that generate serotonin and collagen and that determine gene expression by epigenetic regulation. The identification of many new enzymes that require ascorbate for activity has led to a renaissance of interest in this vitamin, with profound implications for its role in health and disease. It is becoming apparent that the health impact of sufficient vitamin C intake far exceeds the requirement for the prevention of the deficiency disease, scurvy. With newly identified mechanisms

for the prevention and treatment of cancer, and with a demonstrated increase in ascorbate turnover during periods of illness, there is a growing interest in understanding the biochemistry and biology of this essential micronutrient and its impact on human health.

2.2. Pharmacokinetics of Vitamin C

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The absorption, distribution and retention of vitamin C is primarily governed by the family of sodium-dependent vitamin C transporters. The diverse expression and concentration dependency of these transporters throughout the body has resulted in the highly complex, compartmentalized and non-linear pharmacokinetics of vitamin C at physiological levels. Moreover, studies of human sodium-dependent vitamin C transporters (SVCTs) have identified a number of polymorphisms and suggested that these may have significant impact on the pharmacokinetics of vitamin C. In addition to its many biological functions, the putative effect of vitamin C in cancer treatment has been subject of much debate. However, following the critical realization that oral administration of vitamin C profoundly limits the maximum achievable plasma concentration, vitamin C is currently being reinvestigated for its specific toxicity to cancer cells at high concentrations only achievable by intravenous (IV) infusion. Here, the pharmacokinetics of vitamin C appear to change from zero to first order, displaying a constant and dose-independent half-life. The present talk examines the pharmacokinetics of vitamin C under various conditions and discusses its implications for vitamin C homeostasis and sufficiency.

3. Vitamin C Mechanisms of Action

3.1. Vitamin C Cofactor Activities: Vasopressor Synthesis

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Vitamin C is an essential cofactor for a family of metalloenzymes that have numerous biosynthetic and regulatory roles in the body. Research during the 1960–80s determined that these enzymes required vitamin C for the hydroxylation of various biomolecules during the synthesis of collagen, carnitine, catecholamine neuroendocrine hormones and amidated peptide hormones. Decreased collagen synthesis is thought to be responsible for some of the symptoms of the vitamin C deficiency disease scurvy, such as loss of teeth, bruising and poor wound healing, and decreased synthesis of carnitine and neuroendocrine hormones could explain the lethargy, fatigue and depressive symptoms of the disease. Severe infections, such as pneumonia, are a common complication of scurvy and a major cause of mortality in vitamin C-deficient individuals as severe infections can progress to septic multiorgan dysfunction and septic shock. We and others have shown that vitamin C depletion is common during severe infections, requiring gram intravenous doses for repletion of optimal plasma status, and suggesting dramatically enhanced requirements for the vitamin during infections [2]. Recently we proposed that vitamin C-dependent synthesis of the vasopressors norepinephrine and vasopressin may play an important role in supporting cardiovascular function during severe infections and septic shock [3]. Subsequently, several small clinical trials have reported decreased requirements for vasopressor administration (both dose and duration) in patients with severe sepsis and septic shock following administration of gram doses of intravenous vitamin C. We are currently carrying out a pilot randomised controlled trial to confirm these findings and further elucidate the underlying mechanisms of vitamin C, including its role in vasopressor synthesis.

3.2. Regulation of Transcription Factors by Ascorbate

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The transcription factors hypoxia-inducible factors HIF-1 and HIF-2 mediate adaptation of cells to conditions of low oxygen by regulating the expression of hundreds of genes involved in fundamental cellular processes. These processes are conserved across eukaryotes and, in mammals, include cellular metabolism, cell survival pathways, glycolysis and angiogenesis. In cancer, increased HIF transcriptional activity promotes tumour growth and spread, as well as resistance to chemo- and radiation therapy, and it has been associated with poor survival.

HIF transcription factors are heterodimers consisting of oxygen-sensitive subunits and a constitutive α -subunit. Hydroxylation of HIF- α by proline hydroxylases (PHD) targets the protein for degradation, and hydroxylation by factor inhibiting HIF (FIH) leads to its inactivation. Both PHD and FIH enzymes belong to a large family of dioxygenase enzymes that require molecular oxygen and 2-ketoglutarate as substrates, and iron and ascorbate as cofactors. Suboptimal concentrations of either of the substrates or of the cofactors leads to suboptimal dioxygenase activity. An increase in ascorbate therefore reduces levels and activity of HIF.

We hypothesise that optimal intracellular ascorbate levels reduce the aggressive cancer phenotype via regulation of the HIF pathway. We have data ranging from cell culture models, tumour studies in ascorbate-dependent mice, analyses of tumour samples from patients with a range of different cancers and a feasibility study in patients with colorectal cancer that support this hypothesis. Yet, only robust, controlled clinical trials will be able to provide evidence on whether or not ascorbate plays a role in cancer therapy and patient outcome.

3.3. The Epigenetic Role of Vitamin C in Cancer Treatment

Gaofeng Wang

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Genomic loss of 5-hydroxymethylcytosine (5hmC) has been recognized as an epigenetic hallmark of most types of cancer. We showed that vitamin C at 100 M, achievable in vivo by diet and dietary supplements, increased 5hmC content in cultured melanoma cells toward the level of 5hmC in healthy melanocytes, which is associated a shift in the transcriptome and increased apoptosis. Furthermore, we reasoned that vitamin C could change cancer drug responses by altering the epigenome and transcriptome. By high-throughput screening, we identified that vitamin C improved the efficacy of a group of cancer drugs termed bromodomain and extra-terminal inhibitors (BETi). Vitamin C synergistically enhances the efficacy of BETi by reducing H4 acetylation (H4ac), specifically H4K5ac and H4K12ac, via the reduced expression of histone acetyltransferase 1 (HAT1). Co-treatment with vitamin C and JQ1 induced more apoptosis in cultured melanoma cells. Vitamin C deficiency, as modeled in gulonolactone oxidase knockout mice, diminished the treatment outcome of JQ1 for murine melanoma tumour grafts. In contrast, vitamin C supplementation lowered the effective dose of JQ1 needed to successfully inhibit human melanoma xenografts in nude mice. Taken together, dietary vitamin C supplementation could decrease the malignancy of certain cancers and sensitize cancer to chemotherapy such as BETi, thus helping to lessen the severe side effects arising from BETi therapy by reducing the dosage necessary for treatment.

4. Vitamin C and Cancer

4.1. High-Dose Intravenous Vitamin C as a Multi-Targeting Anti-Cancer Agent

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High-dose intravenous ascorbate (IVC) has attracted increasing interests as a low-toxic cancer therapy. IVC bypasses bioavailability barriers of oral ingestion, provides pharmacologic concentrations in tissues, and exhibits selective cytotoxic effects in cancer cells through peroxide formation. The selectivity is related to the mechanisms of action. We postulate that ascorbate-induced reactive oxygen species (ROS) have multiple mechanisms of action that preferably influence cancer cells. First, ascorbate-generated ROS induces DNA damage. Excessive DNA damage induces death in fast dividing cells. Downstream to DNA damage, cellular NAD⁺ decreases as an effect of poly-ADP ribose polymerase (PARP) activation. Decreased NAD⁺ inhibits glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity and depletes ATP in cancer cells, while normal cells maintain their ATP levels. This phenomenon has a root in dysregulated glucose metabolism in cancer cells, known as the Warburg Effect, in which cancer cells depend on a larger proportion on glycolysis for ATP, whereas normal cells depend more on oxidative phosphorylation. Second, lack of NAD⁺ inhibits activity of Sirt-2, a tubulin deacetylase, and therefore increases tubulin acetylation, which in turn disrupts the dynamics of microtubules. This influences cancer cells that are actively undergoing mitosis and migration. Further, ascorbate inhibits the epithelial–mesenchymal transition (EMT), an important process contributing to cancer metastasis. Ascorbate also enhances collagen synthesis in tumour stroma. Despite controversial reports on the effects of elevated collagen in tumour progression, increased collagen by ascorbate treatment was associated with a restriction of tumour invasion in our animal experiments and patients.

Taken together, these data show multi-targeting effects of ascorbate that favour death/inhibition in cancer cells relative to normal cells. With minimal toxicity, the multi-targeting mechanism of ascorbate is advantageous because it could decrease the likelihood of resistance, and provides multiple opportunities for combining with standard chemo and radiation therapies.

4.2. Ovarian and Pancreatic Cancer Trials

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Ovarian Cancer: Yan Ma, Julia Chapman, Mark Levine, Kishore Polireddy, Jeanne Drisko, Qi Chen; Pancreatic Cancer: Kishore Polireddy, Ruochen Dong, Gregory Reed, Jun Yu, Ping Chen, Stephen Williamson, Pierre-Christian Violet, Ziyang Pessetto, Andrew K. Godwin, Fang Fan, Mark Levine, Jeanne A. Drisko, Qi Chen

Pharmacologic ascorbic acid (PAA) as a cancer chemotherapeutic agent remains controversial despite accumulating supportive translational scientific evidence. Our team completed two translational research studies—one of ovarian cancer and the second of pancreatic cancer, with the clinical trials discussed herein. In the first trial in advanced-stage newly diagnosed ovarian cancer, it was shown that PAA induced ovarian cancer cell death *in vitro* and *in vivo*, with concentrations easily achievable by intravenous infusion. Also demonstrated *in vitro* and *in vivo* was synergism of PAA with conventional chemotherapeutic agents, not inhibition of chemotherapy as widely believed. Patients receiving PAA had significant reduction in chemotherapy-associated Grade 1 and Grade 2 toxicities. The studies were not powered to detect efficacy, but showed a trend to benefit when pharmacologic ascorbic was added to conventional chemotherapy. The second clinical trial enrolled pancreatic cancer patients not eligible for surgical resection; a pharmacokinetic study of PAA combined

with gemcitabine chemotherapy was conducted and showed that there was no reduction in gemcitabine or its metabolite. In addition, safety of the combination was confirmed. In this cohort, one participant had reduction in measurable tumour volume and became eligible for surgical resection. With the focus on the Phase I pharmacokinetic analysis of ascorbate with gemcitabine, and the intent to enroll based on the participants needed to determine that effect, the study was not designed to adequately evaluate response in these limited numbers. Advanced-phase clinical trials are now needed to evaluate for the efficacy of PAA.

4.3. Ascorbic Acid and Immune-Recovery after Bone Marrow Stem-Cell Transplantation

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For a long time ascorbic acid (AA) has been believed to be beneficial in treating cancer because of its function as an antioxidant. In the 1970s, Cameron and Pauling reported the increased survival of cancer patients in advanced stages treated with high-dose intravenous ascorbic acid. However, this effect could not be repeated in others. We recently observed that AA that might be beneficial for treatment of cancer: AA is required for T cell differentiation and positively effects in vitro proliferation of T cells and natural killer (NK) cells. Cancer patients receiving stem-cell toxic chemotherapy and haematopoietic stem-cell transplantation (HSCT) have low immunity over a longer period of time due to the reconstitution time of immune cells. We measured significantly lower serum AA levels in these patients. The dietary intake of these patients was often diminished, possibly explaining the low AA values. However, we also observed low plasma AA concentration in patients with acute myeloid leukaemia before the start of treatment, suggesting other mechanisms. Supplementation of AA to these patients could lead to a faster immune system reconstitution by stimulating the proliferation of T and NK cells and the differentiation of T cells. AA supplementation is also easy, cheap and safe at pharmacological levels. At present, a study is being undertaken of patients receiving high-dose chemotherapy plus an autologous stem-cell transplantation to rescue the effect of the high-dose treatment on AA levels and possibly the immune system. This might have a favourable outcome on infections, but also on antitumour responses, since more and more data suggest that the immune system might be crucial in the fight against cancer.

4.4. Outcomes for Stage IV Cancer Patients Treated with High-Dose IV Vitamin C

Damian Wojcik

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Patients with advanced stage IV cancer who have no further standard treatment options available and are referred for hospice palliation often present to integrative medical clinics for advice. Universally they seek improved quality of life (QOL) and, if possible, increased longevity spent with family and loved ones. High-dose intravenous vitamin C (IVC) is frequently offered. Outcomes for our NEHC stage IV cancer patients have been analysed, and will be presented, together with some illustrative cases.

In summary: using the European Organisation for Research and Treatment of Cancer (EORTC) Core 30 QOL questionnaire, a validated instrument used in cancer research for over 30 years, there is a statistically significant overall improvement in the global QOL scores for 71 consecutive stage IV patients receiving IVC infusions. The denominator is the baseline score at $T = 0$, and comparator mean scores were derived from all weekly core 30 questionnaires within each three-month interval for 24 months.

In addition, Kaplan–Meier survival, log survival and cumulative hazard analyses have been performed for a total of 241 consecutive stage IV patients from January 2006 to February 2019,

including 171 treated with IVC infusions, and 70 receiving palliative care only. Both groups had the same age, gender and cancer type distribution. Median survival time improved from 6.2 months for palliative-only patients, to 7.4 months for the IVC patients. There was also a doubling in mean survival time from 7.8 months for palliative patients, to 16.8 months for the IVC patients, (SPSS v 25, Breslow chi square 4.02, df 1, $p = 0.045$), which reflects the long “survival tail” of 5% IVC treated patients who are still alive >5 years.

It is thus appropriate to continue with research to investigate mechanisms by which IVC is able to improve QOL and increase tumouricidal activity. It may be possible in the future to identify subgroups of cancer patients who will best respond to IVC therapy.

5. Vitamin C and Infection

5.1. Historic Overview of Vitamin C and Infection

Harri Hemilä

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Vitamin C was identified in the early 20th century as a substance that was necessary in a certain quantity to prevent scurvy. In early literature, scurvy was linked to pneumonia, implying that vitamin C influences infections. Starting in the 1930s, some German and US physicians proposed that vitamin C is beneficial for treating pneumonia. So far, three controlled trials have reported that vitamin C prevented pneumonia, but the participants of the trials were special, such as schoolchildren and military recruits during WWII.

The effect of vitamin C on the common cold has been studied extensively. Although vitamin C has not prevented colds in the general population, it has halved the incidence of colds in five randomised controlled trials (RCTs) with participants under heavy short-term physical stress. More than 1 g/day of vitamin C shortened the duration of colds by 8% in adults and 18% in children, indicating a physiological effect. Two RCTs compared the efficacy of two different vitamin C doses, and both found that the higher doses, 6 and 8 g/day, were twice as effective in reducing the duration of colds compared with the lower doses of 3 and 4 g/day. Most studies used 1 g/day of vitamin C, which may bias the estimate of efficacy downwards. The practical importance of vitamin C in common cold treatment is open.

The effects of vitamin C are not limited to the common cold and pneumonia. Over 100 animal studies indicate that vitamin C alleviates or prevents infections caused by diverse bacteria, viruses and protozoa [4].

5.2. Intravenous Vitamin C: Pathway to a New Therapy to Save Lives

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Acute lung injury leading to acute respiratory distress syndrome (ARDS) is one of the most common sepsis-associated organ injuries, resulting in significant morbidity and mortality each year. ARDS mortality rates have improved in randomized control trials, but in contemporary pragmatic settings, ARDS mortality has not declined despite advances in care of sepsis patients with ARDS. Bellani et al. reported high in-hospital mortality ranging from 34% to 45%, depending on severity of ARDS. Mortality remains high despite advances in understanding systemic biology and the molecular mechanisms that lead to ARDS. Lung protective ventilation, conservative fluid management and prone positioning are the only widely recommended specific therapies for ARDS. Disease-modifying strategies that target certain mediators to reduce sepsis-induced inflammation leading to ARDS have not improved patient outcomes despite being based on mechanistic research.

Broad spectrum anti-inflammatory agents and other efforts to attenuate lung injury haven't consistently improved outcomes. Prior preclinical and subsequent clinical research performed at Virginia Commonwealth University (VCU) have revealed that high plasma levels of vitamin C act in a "pleiotropic" fashion to attenuate systemic inflammation and correct sepsis-induced coagulation abnormalities, while simultaneously attenuating vascular injury. Padayatty et al. found that high plasma levels of ascorbic acid could only be achieved through intravenous infusion. We have recently performed a National Institutes of Health (NIH)-sponsored phase II multi-center, double blinded, randomized placebo-controlled trial of intravenous vitamin C infusion vs placebo for patients with sepsis-associated ARDS. During the presentation, data from the CITRIS-ALI trial will be presented. These data show that vitamin C was unable to reduce the organ failure score or biomarkers of inflammation or vascular injury. However, vitamin C significantly increased intensive care unit (ICU) free days, hospital free days to day 60 and importantly significantly improved 28 and 60 day survival. The results of CITRIS-ALI may change the way critical care practitioners care for patients with sepsis and ARDS.

5.3. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock

Michael H. Hooper

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Sepsis is a common, deadly manifestation of infections that leads to significant morbidity, mortality and healthcare costs worldwide. The prevalence of vitamin C deficiency is high in septic patients, and administration of intravenous vitamin C has been shown to rapidly replete blood levels. Vitamin C has pluripotent biologic effects. In addition to its well-described antioxidant properties, vitamin C helps preserve vascular endothelial function and supports the biosynthesis of collagen, cortisol, catecholamines and neurotransmitters. In a before–after retrospective analysis of 94 septic patients, we found a strong association between survival and the four-day administration of intravenous (IV) vitamin C 1.5 g q6 h, IV thiamine 200 mg BID and IV hydrocortisone 50 mg q6 h. Patients treated with this regimen had a >30% absolute reduction in mortality despite similar co-morbidities and mortality risk prior to treatment. Improvements in physiologic markers of illness severity suggest that the regimen imparts benefits by improving the physiologic dysregulation of sepsis. The findings in our study are consistent with multiple small trials assessing the effects of vitamin C, thiamine, and hydrocortisone independently.

5.4. The Nurse and Patient Experience of Vitamin C for Sepsis

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Dr. Marik has guided us in several research studies since he came to Sentara Norfolk General Hospital. The one of most importance was his study on the care and treatment of septic patients.

At the onset of this study, a patient was admitted in severe septic shock and requiring life support, a breathing tube, multiple vasopressors, IV fluid, antibiotics and continuous renal replacement therapy dialysis. As usual she was a busy patient and required one-on-one attention to keep her alive. Prepping her family to begin to think about goals for her care and possibly end-of-life wishes, things were grave at best. Dr. Marik decided to administer his vitamin C therapy that consists of a 6-g dose vitamin C infusion, thiamine infusion and IV steroid pushes every 6 h. Within 24 h things started to improve. Vasopressor requirements were lowered, the patient was able to wean off the ventilator and her lab tests were improving. She was extubated and transferred out of the ICU in the coming days.

With great excitement, Dr. Marik kept ordering this cocktail for his septic patients—and it kept working. These patients were getting better, and quickly. The relentless days upon days of us caring for

the same critically ill, septic-shock patients are over so to speak. These patients are getting better within 24–48 h, and we do not have to have nearly as many discussions with families about the patients' final wishes, or have them gather their loved ones to come to say goodbye as we tirelessly and desperately try to keep the patient's body alive to fight their infection just so their families can see them while their heart is beating.

5.5. Vitamin C and Infection in General Practice

Tim Ewer

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Historically, high-dose vitamin C was advocated almost immediately after ascorbic acid was isolated, and the early medical pioneers first published on ascorbate as a prevention and treatment for polio in 1935. This was followed by its use for the inactivation of the diphtheria and tetanus toxins. Between 1943 and 1947, Klenner cured 41 cases of viral pneumonia with vitamin C, and in the 1960s, Cathcart used large doses of vitamin C to treat pneumonia, hepatitis and eventually AIDS.

Since then, the popularity of high-dose vitamin C has grown exponentially, and in a survey of intravenous vitamin C (IVC) use from 2006 to 2008, Padayatty and colleagues reviewed 172 practitioners who had given IVC to a total of 11,233 patients (an average dose of 28 g every four days), and showed that the major indications were for viral infections, cancer and fatigue.

There are numerous studies supporting a variety of mechanisms for the antimicrobial effects of vitamin C, and there is impelling evidence from animal models of sepsis that intravenous ascorbate injections increase survival and protect several microvascular functions. Clinical evidence has been more sporadic and largely related to intensive care experience and some case studies. However, in a growing number of general practice and integrative medicine clinics, IVC is being used effectively to treat a range of infections which are unresponsive to antibiotic therapy. A short overview of these will be presented.

6. Poster Abstracts

6.1. Pharmacologic Vitamin C Improves Fatigue and Quality of Life in Cancer Patients

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High-dose vitamin C has been administered by health care practitioners for many decades as a complementary and alternative therapy for numerous conditions, including cancer, infection and fatigue. We have carried out a number of case studies investigating the quality of life of cancer patients receiving chemotherapy or undergoing palliative care using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ). This assesses the common cancer-related symptoms of fatigue, nausea/vomiting, pain, dyspnoea, insomnia, appetite loss, constipation and diarrhoea. This questionnaire also assesses physical, role, emotional, cognitive and social functioning, and has a global health status score. Because fatigue is one of the most common and debilitating symptoms reported by cancer patients, we also used the Multidimensional Fatigue Symptom Inventory (MFSI) which assesses multiple aspects of fatigue (i.e., general, physical, emotional and mental fatigue, as well as vigour), and has a total fatigue score. Here we report on two cases, an 81-year-old male undergoing palliative care for an inoperable pulmonary angiosarcoma, and a 45-year-old female undergoing chemotherapy for invasive ductal carcinoma of the breast (ER+, PR+, HER2-). The first case received 30 g/d IV vitamin C for seven days, while the second case received 50 g/d IV vitamin C twice a week for four weeks [5,6]. The quality of life (EORTC) and fatigue (MFSI) questionnaires were administered before and after the vitamin C interventions.

We observed a decrease in the symptoms of fatigue, nausea, pain, insomnia and appetite loss following vitamin C administration in both cases, as well as an increase in physical, role, cognitive and social functioning, and an improvement in overall health status. With respect to the multiple aspects of fatigue, decreases in general, physical, emotional and mental fatigue were observed. No adverse side effects were reported by the patients or clinical staff. Appropriately designed placebo-controlled trials to confirm the quality of life effects of intravenous vitamin C in cancer patients are warranted.

6.2. No Reported Renal Stones with Intravenous Vitamin C Administration: A Prospective Case Series Study

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A few cases associating high-dose intravenous vitamin C (IVC) administration with renal stone formation have been reported in the literature; however, no long-term studies investigating IVC administration and reported renal stones have been carried out. Our aim was to measure the frequency of reported renal stones in patients receiving IVC therapy. We carried out a prospective case series study of 157 adult patients who commenced IVC therapy at Integrated Health Options clinic between 1 September 2011 and 31 August 2012, with a follow-up at 12 months. Inquiries into the occurrence of renal stones were conducted at enrollment, 6 and 12 months, and renal function blood tests were conducted at enrollment, 4 weeks and every 12 weeks thereafter in a subgroup of patients. No renal stones were reported by any patients in the study, despite 8% of the patients having a history of renal stones. In addition, the majority of patients investigated had stable renal function during the study period as evidenced by little change in serum creatinine levels and estimated glomerular filtration rate (eGFR) following IVC. In conclusion, IVC therapy was not associated with patient-reported renal stones. Although not the primary focus of this study, it was also observed that there was no significant change in mean serum creatinine or eGFR for those who had follow-up renal function blood tests [7].

6.3. The Use of Intravenous Vitamin C as a Supportive Therapy for a Patient with Glioblastoma Multiforme

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Glioblastoma multiforme is a high-grade malignant brain tumour with a poor prognosis. Here we report the case of a woman with glioblastoma who lived for over four years from diagnosis (median survival 12 months and 2% survival for three years), experiencing good quality of life for most of that time. She underwent initial debulking craniotomy, radiotherapy and chemotherapy, as well as having intravenous vitamin C infusions two to three times weekly over the four years from diagnosis. Her progress was monitored by blood tests, regular computerised tomography (CT) and magnetic resonance imaging (MRI) scans, clinical reviews and European Organization for the Research and Treatment of Cancer quality of life questionnaires (EORTC QLQ C30). Our case report highlights the benefits of intravenous vitamin C as a supportive therapy for patients with glioblastoma [8].

6.4. Intravenous Vitamin C Administration Improved Blood Cell Counts and Health-Related Quality of Life of Patient with History of Relapsed Acute Myeloid Leukaemia

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A 52-year-old female presented to Integrated Health Options clinic in October 2014 with a history of relapsed acute myeloid leukaemia (AML, diagnosed in 2009 and relapsed in 2014).

Intravenous (IV) vitamin C therapy was initiated (in 2014) following completion of chemotherapy as an alternative to haematopoietic stem-cell transplantation. IV vitamin C was administered twice weekly at a dose of 70 g/infusion. Within four weeks of initiation of IV vitamin C therapy, there was a dramatic improvement in the patient's blood indices, with platelet cell counts increasing from $25 \times 10^9/L$ to $196 \times 10^9/L$, and white blood cell counts increasing from $0.29 \times 10^9/L$ to $4.0 \times 10^9/L$, with further improvements observed over the next 18 months. Furthermore, there was a clear and sustained improvement in the patient's health-related quality of life scores assessed using a validated questionnaire. She has remained healthy and in complete remission until the present day. This case study highlights the benefits of IV vitamin C as a supportive therapy for previously relapsed AML [9].

6.5. Remission of Metastatic Prostate Cancer with Integrative Treatment: Five-Year Follow-Up

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A 53-year-old police officer was diagnosed with progressive prostate malignancy in 2014 following 18 months of pelvic pain and radical prostatectomy. MRI confirmed spinal, meso-rectal and seminal vesical involvement with additional infiltration of the S3 nerve root. Pelvic radio-therapy, initially deemed too risky, was given for palliation in 2015.

Integrative management:

1. Intravenous vitamin C. Following consultation in May 2014, twice-weekly intravenous vitamin C (IVC) was started combined with 6 g daily by mouth. The IVC dose was increased to 50 grams once a normal G6PD was confirmed. Twice-weekly IVC was maintained for seven months and then weekly for a further five months, by which time the patient was pain-free and cleared by the oncologist.
2. Dental aspects. Nine amalgam fillings (18 surfaces) and a gold crown were present. Following discussion, protected amalgam replacement was completed between June and November 2014.

MRI report (November 2014), revealed no evidence of cancer. To date, the patient's prostate-specific antigen (PSA) remains undetectable. He remains well and has been in full-time police duties since 2017.

6.6. Ascorbate Targets Acute Myeloid Leukaemia Subclones with Mutations that Affect TET2 Activity

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Heterozygous mutations in TET2, WT1, IDH1 and IDH2 occur in 30%–50% of patients with AML. These mutations are largely mutually exclusive and result in compromised activity of TET2, an ascorbate-dependent DNA demethylase. Pre-clinical models of AML with mutations in IDH or TET2 have shown that ascorbate treatment normalizes leukemic cell function through the optimization of residual TET2 activity. To date, no clinical studies have been published demonstrating the benefit of ascorbate for patients with these mutations. We investigated the genetic evolution of AML in a patient where the disease was refractory to chemotherapy and where subsequent ascorbate treatment was

associated with a 2.5-year clinical remission. We carried out whole exome sequencing (WES) of the patient's samples to determine AML subclones at diagnosis, remission and relapse. WES at diagnosis revealed mutations in four known AML driver genes (DNMT3A, TET2, WT1 and NPM1), which were absent in the remission sample. With the exception of the WT1 variant, these mutations resurfaced at relapse along with mutations not present at diagnosis. Our data suggest that the TET2 and WT1 mutations arose in separate clones, and that the WT1 mutant clone was more sensitive to treatment. Together with preclinical findings, this investigation provides support for the hypothesis that ascorbate could be beneficial for treating AML where mutations result in decreased TET2 activity.

6.7. Effect of Ascorbate on Tumour-Associated Macrophage Phenotype Ex Vivo

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Increasing evidence shows a beneficial role for ascorbate in cancer treatment. Currently, only ascorbate-mediated cancer cell toxicity or tumour characteristics have been considered, and not the effect on immune cells. Circulating leukocytes have very high intracellular ascorbate levels, and this drops in cancer patients. Therefore, we investigated the effect of ascorbate on immune cells in the context of the tumour microenvironment.

Here, we studied the effect of ascorbate supplementation in an ex vivo model of tumour-associated macrophages/monocytes. Primary murine bone marrow monocytes were isolated and grown with Lewis lung carcinoma cell-conditioned media (LLCM) (40% v/v) for six days with or without ascorbate supplementation (500 μ m). Then, overnight monocyte-conditioned media was harvested for ELISA measurements. Additionally, monocytes were stimulated for 24 h with tumour microenvironment stimuli such as hypoxia (1% O₂) or crude tumour cell lysate (to mimic alarmins from necrotic tumour cells).

After seven days of culture in LLCM, monocytes attained a spindle shape associated with an M2 phenotype. M2 proteins, VEGF and TGF- accumulated in media of LLCM-differentiated monocytes. Ascorbate-supplemented monocytes were less spindle and had lower VEGF and TGF-secretion. Hypoxia increased VEGF and TGF- secretion, and this too decreased with ascorbate supplementation. Tumour cell lysate stimulation did not alter VEGF or TGF- levels, but increased IL-6 secretion. VEGF and TGF- levels decreased and IL-6 levels increased with ascorbate supplementation.

These results suggest that high ascorbate levels in monocytes may dampen the pro-tumour monocyte phenotype. Further studies will determine whether ascorbate affects the function of these cells.

6.8. What's Hairy and Has up to 14 Times as Much Vitamin C as a Lemon?

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Kiwifruit are already renowned for their high vitamin C content. Fruit of the familiar green Zespri Green kiwifruit contain about 80 mg/100 g fresh weight (FW), and Zespri SunGold kiwifruit about 160 mg/100 g FW, but there is potential to breed kiwifruit cultivars with much more vitamin C. *Actinidia eriantha* is a species related to the familiar commercial kiwifruit *A. chinensis*. The fruit of *A. eriantha* have dark green flesh and are covered in white hair. The mean vitamin C content of *A. eriantha* fruit is about 720 mg/100 g fresh weight—that's about 14 times more than a lemon and about 90 times more than an apple! Breeders have crossed *A. eriantha* with *A. chinensis* to produce hybrids with very high vitamin C. Breeding is being supported by research on the biosynthetic pathways of vitamin C and through the use of new technologies. A DNA marker linked to a supergene controlling the high vitamin C in *A. eriantha* hybrids has been identified, and an efficient technique for estimating the vitamin C content of fruit from large numbers of seedlings has been developed. Its high vitamin

C content and other nutritional components, including folate, dietary fibre and other vitamins and minerals, combined with its good flavour, make kiwifruit an attractive natural alternative to vitamin C pills for supporting health needs.

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