Vitamin C and cancer risk and treatment

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Abstract

Vitamin C (L-ascorbic acid) works as a strong reductant, radical scavenger, and protector of cell membranes against primary peroxidative damage in tissues and in the extracellular fluid. L-ascorbic acid is involved in the synthesis of collagen and many other biologically relevant substances, enzyme activity, xenobiotic detoxification, and prevention of forming carcinogenic nitrosamines. It also plays a role in the immune system. Numerous data indicate that cancer patients suffer from vitamin C deficiency. Studies show that people with a low vitamin C intake have an increased risk of head and neck cancers as well as lung, gastric, pancreatic, cervical, rectal, or breast cancer. On the other hand, there is no clinical evidence to support the thesis that antioxidant supplements (including vitamin C) prevent cancer. Observational trials investigating high doses of intravenous L-ascorbic acid in previously treated cancer patients have shown that it allows an increase in quality of life and may improve physical, mental, and emotional functions, as well as reducing adverse effects of standard anticancer treatment, including fatigue, nausea, vomiting, and appetite loss. So far, there were a few randomized controlled trials and they have not reported any statistically significant improvements in the overall or progression-free survival with vitamin C, as compared to the control arm. However, preclinical data indicating a role of L-ascorbic acid in modulation of immune response and its involvement in epigenome remodeling suggest its new potential clinical applications in cancer patients, especially in combination with immunotherapy. It seems reasonable to further investigate the value of vitamin C as a supportive treatment or in combination with anti-cancer targeted therapy.

Keywords

Vitamin C • L-ascorbic acid • cancer • carcinogenesis • immunotherapy

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Introduction: biochemistry and metabolism

Vitamin C (L-ascorbic acid) is a small, highly water-soluble molecule present in all plants, and also in most animals [1, 2, 3] which synthesize it in the liver or the kidneys. It is derived from glucose and its molecular structure resembles that of the substrate. Humans cannot synthesize vitamin C due to the mutationally inactivated L-gulonolactone oxidase gene (GULO gene). It is coding for the enzyme responsible for the final step in the compound biosynthesis. Other primates, caviar and fruit bats also rely on dietary intake of vitamin C [4]. Despite the fact that L-ascorbic acid is readily acquired and distributed in the human body, it cannot be stored. Its

turnover is usually constant, however, it can be accelerated in disease processes and oxidative stress periods.

In the extracellular fluid, L-ascorbic acid is the most important antioxidant. It is present in a number of tissues including the eye lens, liver, pituitary gland [5]. In the brain, the adrenal gland and white blood cells its concentration may reach up to 20 mM [4].

L-ascorbic acid works as a strong reductant, radical scavenger, and protector of cell membranes against primary peroxidative damage. It reduces unstable oxygen, nitrogen, and sulphur-centered radicals. Data indicate that in vitro, it is preferentially oxidized in plasma before other antioxidants [2]. An antioxidant property of L-ascorbic acid is connected with its ability to readily undergo one- or two-electron oxidation

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and generate the ascorbyl radical or L-dehydroascorbic acid, respectively. The oxidized form of vitamin C is unstable at neutral pH. In vivo, owing to its reduction by glutathione or thioredoxin, L-dehydroascorbic acid is regenerated to reduced vitamin C. Otherwise, it rapidly decomposes to diketogulonic, oxalic, and threonic acids [4]. Vitamin C acts synergistically with vitamin E, which is an important lipid-soluble antioxidant, by enhancing or reinstating its activity [5]. Vitamin E antioxidant activity in cell membranes generates the tocopheroxyl (chromanoxyl) radical. Vitamin C reacts with it and, at the same time, revives tocopherol and transfers the oxidative challenge to the aqueous phase.

There are data that L-ascorbic acid can increase cell respiration and adenosine triphosphate (ATP) production in osteoblasts [5].

L-ascorbic acid accelerates hydroxylation reactions in numerous pathways by directly or indirectly providing electrons to enzymes that require prosthetic metal ions in a reduced form to accomplish full enzymatic activity [5, 6]. Vitamin C augments absorption of non-heme iron in the intestine [7].

As a cofactor for prolyl and lysyl hydroxylase enzymes, vitamin C is involved in the synthesis of collagen. It is also engaged in the synthesis of carnitine and neurotransmitters, cytochrome P-450 activity, metabolism of cholesterol and tyrosine. Additionally, there are data on L-ascorbic acid's involvement in the activity of cholecystokinin, oxytocin, vasopressin, and alpha-melanotropin [7].

Vitamin C takes part in detoxification of xenobiotics, reduces nitrates, and prevents the formation of carcinogenic nitrosamines [5]. Interestingly, there are data indicating different roles L-ascorbic acid plays in the immune system. It is involved in strengthening of leukocyte chemotaxis, interferon production and complement C1q activity. Vitamin

C is required for active phagocytosis. These facts are in agreement with relatively high concentrations of vitamin C observed in lymphocytes as compared to other cells. Vitamin C is also engaged in humoral immunocompetence because it is essential for immunoglobulin production [5, 6]. Figure 1a. diagrammatically summarizes the influence of L-ascorbic acid on metabolism in healthy individuals.

[Fig. 1]

A total normal human body content of L-ascorbic acid is up to 2 g. Its normal range in blood plasma, where it is bound by albumin, produced by intake of vitamin C-rich foods (fruits and vegetables) is from 0.70 to 1.4 mg/dL, i.e. 0.04–0.08 mM (unit conversion 1 mg/dL = 0.05678 mM) and it can reach 0.15 mM by higher intake from dietary supplements [7, 8]. Fasting L-ascorbic acid blood levels are recognized as representative for body content. However, there is an opinion that the level of vitamin C should be measured in leukocytes rather than in the blood [2]. It is leukocytes that represent a target tissue for L-ascorbic acid, and its level in leukocytes is more reliable as it is not affected by recent dietary intake of the nutrient. The body storage and bioavailability of vitamin C depend on intestinal absorption, tissue capacity, renal reabsorption and excretion, and accompanying diseases. Intestinal (small intestine) and renal (proximal tubules) absorption takes place via sodium-dependent transporters SVCT1 (encoded by *SLC23A1* and mapped to 5q31.2) and SVCT2 (encoded by *SLC23A2* and mapped to 20p13) [9]. The maximal serum level of vitamin C that can be achieved by oral intake of its formulations ranges from 1.3 to 4.0 mg/dL (0.074–0.237 mM), while intravenous infusions may increase its concentration to more than 350 mg/dL (20–49 mM) [7]. Elevated blood level of L-ascorbic acid is rapidly cleared by the renal system, proportionally to concentration.

Figure 1a.

Figure 1b.

Figure 1. Schematic summary of L-ascorbic acid influence on metabolism in healthy individuals (a) and its impact on cancer cells and host metabolism in cancer patients (b).

Vitamin C is transported to different tissue cells through active uptake by the above-mentioned sodium-dependent transporters SVCT1 and SVCT2 [6], so its tissue concentration may be many times higher than plasma levels. The same transporters are used by blood cells, except red blood cells to which oxidized forms of vitamin C (L-dehydroascorbic acid) are delivered through GLUT1, 3 and 4. GLUTs are also expressed in other blood cells [6]. Extracellular vitamin C is oxidized, transported in the oxidized form, and reduced to L-ascorbic acid which is trapped intracellularly [10]. In other words, vitamin C is delivered to white blood cells through the activity of different transporters, that is, SVCT 1 and SVCT2, for reduced vitamin C form, and GLUT1, 3, and 4 for its oxidized form [6].

Cancer cells are metabolically reprogrammed and have an increased demand for glucose and higher glycolytic metabolism which are compensated with an amplified quantity of glucose transporters [11]. GLUTs and glycolysis genes are positively regulated by HIF-1 in hypoxic, low-nutrient, and high-growth conditions. In normal tissue, much higher concentrations of glucose make GLUTs only slightly involved in the uptake of vitamin C; however, in cancer cells the overexpression of GLUTs overcomes the inhibitory effects of glucose and results in increased L-ascorbic acid accumulation through uptake of L-dehydroascorbic acid [6]. Ovarian cancer patients overexpressing GLUT-1 are characterized by a poorer prognosis [11]. GLUT1 overexpression in colorectal cancer cells and CRCs and *KRAS* or *BRAF* mutations correlate with glucose uptake measured by 18-fluorodeoxyglucose positron emission tomography (FDG-PET) [12]. On the other hand, genetic variations in *SVCT-2* were proved to be closely associated with the risk of various cancers, including gastric cancer, lymphoma, and head and neck squamous cell carcinomas [13]. Lv and colleagues revealed that *SVCT-2* was highly expressed in hepatocellular carcinoma (HCC) samples as compared to peritumor tissues, *SVCT-2* expression was positively correlated with stemness-related genes, and high expression of *SVCT-2* in clinical tumor samples from cohorts of HCC patients correlated with shorter overall survival (OS).

Inadequate intake of L-ascorbic acid results in low plasma concentrations, which are likely to correlate with poor tissue saturations [6]. Extreme deficiency may lead to scurvy, which is a life-threatening condition. Vitamin C depletion occurs when its blood level falls below 0.5 mg/dL (0.028 mM) [5]. Scurvy usually develops within 1–3 months of cessation of vitamin C intake and when its level is lower than 0.2 mg/dL (0.011 mM) [7]. The disease is well known and manifested by generalized tissue disintegration at all levels, involving the dissolution of intercellular ground substance, the disruption of collagen bundles, and the lysis of the interepithelial and interendothelial cement. Interestingly, in patients with scurvy,

generalized undifferentiated cellular proliferation throughout the reversion of tissue to a primitive form was observed [14]. As little as 10 mg of vitamin C per day prevents scurvy; this is easily obtained with a balanced diet in healthy individuals. The recommended daily intake of vitamin C is 200 mg and it maintains an optimal plasma concentration of 0.07 mM. Unfortunately, it is estimated that 10% of adults in the United States have plasma concentration of L-ascorbic acid <0.023 mM [14].

The risk of vitamin C deficiency is increased in individuals suffering from chronic diseases and depression, as well as in hospitalized and post-operative patients and smokers [15]. Blood L-ascorbic levels are also lowered in people consuming acetylsalicylic acid and oral contraceptives and those exposed to various types of stress [16].

Among people who are at risk of developing vitamin C deficiency, cancer patients are of special interest since they usually present severe deficiency of the vitamin. As a chronic disease, cancer is associated not only with an inadequate intake of L-ascorbic acid but also with an increased turnover. Furthermore, numerous theories suggest that vitamin C deficiency plays a significant role in carcinogenesis, and emphasize the importance of high-dose intravenous administration of L-ascorbic acid in palliation and anti-cancer treatment. This review summarizes current clinical knowledge in these areas.

Decreased daily vitamin C intake and risk of cancer

There is a theory explaining a potential role of L-ascorbic acid deficiency in carcinogenesis. It suggests a leakage of damaged DNA and/or mutated DNA to cytosol caused by both endogenous and exogenous risk factors, such as ageing-induced reactive oxygen specimens (ROS), mitochondrial dysfunction, smoking, or reduced genome stability due to reduced Ten-Eleven Translocation (TET) family enzymes activity [17]. If vitamin C deficiency occurs, they can activate the innate immunity cytosolic DNA-sensing cGAS-STING pathway and cause inflammation (e.g., IL-6 generation), cellular senescence and cancer.

Some studies indicate the association of decreased vitamin C intake with increased risk of developing cancer. It indirectly implies its role in carcinogenesis.

The historical view on the impact of vitamin C intake on cancer risk is neatly summarized by Block et al. The authors published a critical review of case-control and prospective studies carried out before 1991 [16]. They were aimed at evaluating a potential beneficial effect of L-ascorbic acid in daily diet on cancer prevention. Six of seven case-control studies (most of them included controls for smoking and

alcohol intake) reported significant protective effects of increased L-ascorbic acid intake or fruit intake against oral cancer. Individuals with the lowest intake of vitamin C or fresh fruit had a risk ratio of 1.7-2.0 times higher than those whose diet provided the nutrient on a regular basis. In six studies, a low intake of vitamin C was associated with a risk ratio of 2.0-2.4 for laryngeal cancer and esophageal cancer after control assessment for potential confounding factors, such as alcohol intake and smoking. Also, a protective effect preventing the upper aerodigestive tract cancer onset was observed in the case of vegetable consumption (twofold risk in nondaily vs. daily consumers) [18]. In the studies reviewed by Block, different fruits, especially citrus fruits and juices, were identified as the only significantly protective foods preventing esophageal cancer. In a more recent paper, by comparing the highest vs. the lowest categories of vitamin C intake, Bo et al. found that vitamin C intake was inversely associated with the risk of esophageal cancer [overall OR 0.58, 95% CI 0.49-0.68, $I^2=56\%$]. They revealed a linear dose-response relationship [19].

Other studies included in the review by Block indicate dietary L-ascorbic acid intake and vegetable consumption as statistically significant protective factors in lung cancer prevention. According to Fontham et al., individuals consuming < 90 mg of vitamin C per day had lung cancer risk estimates of 1.5 ($P < 0.001$) after adjusting for smoking, income, and other confounders as compared those who consumed 140 mg or more [20]. If vitamin C intake was < 50 mg/d, the relative risk of lung cancer was 4.3 times higher than in people with a higher intake. According to Block, vitamin C and fresh fruit and vegetable intake also protects against pancreatic cancer, since individuals consuming small amounts of vitamin C or fresh fruit and vegetables had a relative pancreatic cancer risk between 1.4 and 2.6. On the other hand, in their recent study, Hua et al. concluded that there was insufficient evidence to find any relationship between vitamin C intake and risk of pancreatic cancer. The strong inverse association observed in case-control studies (0.58, 95% CI: 0.52-0.66) could be affected by biases (e.g., recall and selection biases) [21].

A statistically significant protective effect of L-ascorbic acid or fruit consumption preventing gastric cancer was also described in the Block's paper. Individuals with the lowest intake of fresh fruit or vitamin C showed a risk of developing gastric cancer approximately two (White people) or three times (Black people) higher than those with the greatest consumption. There is a hypothesis that the anti-nitrosation mechanism lies at the basis of L-ascorbic acid protection against stomach cancer. Researchers have found that concentration of vitamin C in the gastric juice of people without any pathologic changes in the stomach is three times higher than that in the plasma; not, however, in patients with chronic

gastritis. Moreover, in healthy controls, L-ascorbic acid occurs predominantly in the reduced form which facilitates the anti-nitrosation reaction, whereas in patients with gastritis it is mostly present in the oxidized form. The inverse association of vitamin-C dietary intake and gastric cancer was also confirmed by Li et al. in their recent study [22]. Nevertheless, they also observed no such association for blood levels of vitamin C. Also, in their modern study, Hoang et al. [23] observed that vitamin C and vitamin C-contributing foods may have a protective effect by preventing gastric cancer. They included 830 control subjects and 415 patients, and collected data on demographics, medical history, lifestyle, and dietary and nutrient intake.

Most of the studies dedicated to a potential effect of L-ascorbic acid on cervical cancer reviewed by Block detected statistically significant, approximately two times higher, protective effects of its increased intake. It appeared that fruit juice consumption also lowered the risk of cervical cancer. The preventive effects of vitamin C intake on cervical neoplasms in case-control studies were confirmed in a more up-to-date meta-analysis by Myung et al. [24].

When focusing on the association between L-ascorbic acid, fruit, or raw vegetable intake and rectal cancer, Block found statistically significant results. The risk of rectal cancer associated with low intake of vitamin C or its dietary sources was approximately 1.7 among men, and even higher (3.3) among women. Although in the case of colon cancer, data were not consistent, still most of the reviewed studies revealed a statistically significant protective effect of vitamin C or vitamin C-rich foods [16].

Inhibiting nitrosation by L-ascorbic acid in humans can impact the development of bladder tumors but none of the four studies reviewed by Block indicated its statistically significant protective effect. Block cited the report by Thomas Sinks and John R Wilkins III (1989) revealing a statistically significant threefold increase in the risk of childhood brain tumor in the case of low maternal intake of vitamin C during pregnancy. According to Block, L-ascorbic acid intake did not affect the risk of ovarian, endometrial or prostate cancer. However, some further investigations have suggested an increased risk of prostate cancer associated with increased vitamin A and vitamin C intake in some circumstances [22]. An opposite conclusion was drawn by Bai et al. in their modern meta-analysis. They found that intake of vitamin C deriving from food was inversely associated with prostate cancer risk (RR 0.91; 95%CI: 0.84-0.98, $p = 0.018$) [25].

A meta-analysis by Howe et al. revealed that L-ascorbic acid intake had a statistically significant inverse association with breast cancer risk (relative risk for highest vs. lowest quintile, 0.69; $p < 0.0001$) [15]. Also a large, prospective study by Zhang et al. [26] which aimed at evaluation of long-term intakes of vitamins A, C, and E and breast cancer risk revealed

that consumption of fruits and vegetables rich in specific carotenoids and vitamins may reduce premenopausal breast cancer risk. However, these results have not been confirmed by the more up-to-date meta-analysis by Fulan *et al.* [27] focused on the associations between retinol, vitamins A, C, and E intake, and breast cancer risk. The authors have found that both the total intake of vitamin A and retinol can reduce breast cancer risk, but they have not identified any associations between other vitamins and breast cancer. Also, a modern study by Cui *et al.* [28] proved that dietary vitamin C was not associated with breast cancer.

In a prospective study, Eichholzer *et al.* measured plasma L-ascorbic acid, vitamin E, retinol, and carotene levels in 2,974 men working in Basel, Switzerland. After a 17-year follow-up, the vital status of all the participants was assessed [29]. A total of 290 men died from cancer, including 87 with lung cancer, 30 with prostate cancer, 28 with stomach cancer, and 22 with colon cancer. The overall cancer death rate was associated with low mean plasma levels of carotene (adjusted for cholesterol) and of vitamin C. After calculation of the relative risk based on the Cox model and excluding mortality during the first two years of the follow-up period, low levels of plasma vitamin C and lipid-adjusted vitamin E were associated with a significantly increased risk of lung cancer. Four years later, Loria *et al.* published a similarly designed prospective study [30]. Serum vitamin C concentrations were measured in adults as a part of the second National Health and Nutrition Examination Survey (1976-1980) which collected extensive demographic, medical history, nutritional, clinical, and laboratory data on a multistage, probability sample of the civilian, non-institutionalized US population. Vital status was ascertained 12–16 years later. The study revealed that men in the lowest (< 0.0284 mM) compared with the highest (≥ 0.0738 mM) serum vitamin C quartile had a 57% higher risk of dying from any cause (RR = 1.57; 95% CI: 1.21–2.03) and a 62% higher risk of dying from cancer (RR = 1.62; 95% CI: 1.01–2.59). Interestingly, there was no association between serum vitamin C and mortality among women. Those findings were consistent when analyses were limited to nonsmokers or adults who never smoked, which suggested that the observed relations were not related to smoking. In contrast, the associations among male smokers were generally attenuated and not significant. Cancer deaths among men were clustered in the respiratory and digestive organs, whereas among women, deaths were more evenly distributed among sites. Stable risk estimates were possible only for lung cancer among men – those in the lowest quartile had a higher risk of dying from lung cancer (RR = 2.97; 95% CI: 1.46–6.08) than those in the highest quartile.

The impact of vitamin C supplementation on cancer risk

Numerous up-to-date meta-analyses are focused on vitamin C supplement intake and cancer risk.

The efficacy of specific L-ascorbic acid supplementation in prevention of cancer was investigated by Lee *et al.* [22]. The meta-analysis included seven trials (62,619 participants; 31,326 and 31,293 were randomized to L-ascorbic acid supplementation and control or placebo groups, respectively). They revealed no significant association between vitamin C supplementation and cancer (RR = 1.00; 95% CI: 0.95–1.05). A subgroup meta-analysis based on a dose of L-ascorbic acid administered, a follow-up period, methodological quality, cancer mortality, gender, smoking status, country, and type of cancer also showed no efficacy for cancer prevention.

Lin *et al.* [31] followed up 7627 women who were randomly assigned in the Women's Antioxidant Cardiovascular Study, a double-blind, placebo-controlled $2 \times 2 \times 2$ factorial trial of vitamin C (500 mg of ascorbic acid daily), natural-source vitamin E (600 IU of α -tocopherol every other day), and beta carotene (50 mg every other day) and were free of cancer before random assignment. The authors revealed that supplementation with vitamin C, vitamin E, or beta carotene offered no overall benefits in the primary prevention of total cancer incidence or mortality.

In 2010 Myung *et al.* [32] published their work on effects of antioxidant supplements on cancer prevention. This meta-analysis of 22 RCTs included 161,045 subjects (88,610 in antioxidant supplement groups and 72,435 in placebo or no-intervention groups). They showed that antioxidant supplements had no preventive effect on cancer (RR = 0.99; 95% CI: 0.96–1.03), regardless of whether it was primary or secondary prevention. Subgroup analyses revealed no preventive effect on cancer according to type of antioxidant, type of cancer, or the methodological quality of the studies. A subgroup meta-analysis of four trials revealed that the use of antioxidant supplements significantly increased the risk of bladder cancer (RR = 1.52; 95% CI: 1.06–2.17). That phenomenon was further investigated in a meta-analysis by Park *et al.* [33]. They included 14 randomized controlled trials (RCTs) in the final analysis and revealed that vitamin and antioxidant supplements showed no preventive effect on bladder cancer (RR = 1.04; 95% CI: 0.92–1.17; $I^2 = 39.7\%$). Moreover, beta-carotene supplementation alone marginally increased the risk of bladder cancer (RR = 1.44; 95% CI: 1.00–2.09; $I^2 = 0.0\%$; $n = 3$).

In 2010, Jiang *et al.* published a meta-analysis on the efficacy of antioxidant vitamins and selenium supplementation in prostate cancer prevention [34]. It covered nine RCTs with 165,056 participants and showed no significant effects of

supplementation with beta-carotene, L-ascorbic acid (RR = 0.98, 95% CI: 0.91–1.06) (2 trials), vitamin E, or selenium versus placebo on prostate cancer incidence. The mortality of prostate cancer was not related to supplementation of the above-mentioned antioxidants either.

Myung was also a coauthor of another meta-analysis dedicated to efficacy of vitamin and antioxidant supplements in prevention of esophageal cancer [35]. Again, meta-analysis of 10 trials showed no benefits of such supplements for prevention of esophageal cancer (RR = 1.04; 95% CI: 0.86–1.25; I²=0.0%). Also, subgroup meta-analyses revealed that vitamin and antioxidant supplements had no preventive efficacy on esophageal cancer, either in the high risk or in non-high risk groups.

Pais and Dumitrașcu focused on colorectal cancer prevention with antioxidants [36]. They included 20 RCTs (26,859 participants) out of which 12 analyzed colorectal cancer incidence, whereas the remaining 8 analyzed colorectal adenoma recurrence. Antioxidant supplements had no significant effect on colorectal cancer incidence or colorectal adenoma recurrence (RR = 0.94, 95% CI: 0.84–1.06, *p* = 0.32). They had no significant impact on overall mortality (RR = 1.03, 95% CI: 0.99–1.07, *p* = 0.12) or cancer related mortality (RR = 1.05, 95% CI: 0.94–1.16, *p* = 0.38) either. Vitamin C and Vitamin E combination slightly reduced colorectal cancer incidence with no effect on overall mortality. Similar data came from a meta-analysis by Bjelakovic et al. which was dedicated to the impact of anti-oxidative vitamin (L-ascorbic acid among others) supplementation on the risk of colon adenoma [37]. They reviewed eight RCTs published before October 2005 (17,620 individuals). The authors concluded that there were no clear-cut data indicating that supplementation with beta-carotene, vitamins A, C, E, or selenium applied in different doses and combinations affects the risk of colon adenoma.

Cadeau et al. [38] estimated vitamin C intake from foods and vitamin C supplement use in 57,403 postmenopausal women from the Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale (E3N) prospective cohort in the years 1995–2005. The authors observed that vitamin C supplement use was associated with increased postmenopausal breast cancer risk in women with high vitamin C intake from foods. Moreover, the above-mentioned study by Cui et al. also revealed that total and supplemental vitamin C intake had weak overall positive associations with breast cancer [28].

According to Chang et al., vitamin treatment or supplements with antioxidant properties were found to have no preventive effect on skin cancer [39]. However, in a more recent review, Katta and Brown reported that although supplements had not shown efficacy, intake of antioxidants via consumption of whole foods could bring potential health

benefits. For example, in a prospective observational study of 1360 adults followed over the course of 11 years, consumption of vegetables and fruits was associated with a 54% decrease in the risk of squamous cell carcinoma [40].

To sum up, the meta-analyses of RCTs demonstrated no clear clinical evidence to support the preventive effect of antioxidant supplements (including L-ascorbic acid) on cancer. Moreover, certain data indicate that use of some of them may even be harmful. We assume that the disagreement between different studies evaluating the role of inadequate uptake of L-ascorbic acid and its supplementation may result from the fact that uptake of the nutrient is highly dose-dependent. Subjects already saturated with vitamin C through their daily diet are very unlikely to benefit from supplementation.

It is also worth mentioning that vitamin C status may vary by race. Kant et al. used data from the NHANES III (*n* = 13113) and NHANES 1999–2002 (*n* = 7246) to examine, among other factors, ethnic differentials in serum concentrations of nutrients of putative public health importance in American adults [41]. They revealed that the education- and income-adjusted serum vitamin C concentration in non-Hispanic black people was lower as compared to non-Hispanic white population. Vitamin C profiles among Mexican-Americans were better than those of non-Hispanic white people. On the other hand, Suarez et al. did not find race differences in the relation of serum vitamin C levels in 176 non-smoking, healthy, white and African American adults [42]. Nevertheless, race significantly moderated the relation of vitamin C to leukocyte count, with a lower vitamin C level being associated with a higher leukocyte count in African Americans only, not in white Americans. The authors raise an important and still unanswered question: whether lower levels of micronutrients contribute uniquely to racial health disparities. Globally, the prevalence of vitamin C deficiency appeared to be highest among South Asians, which might contribute to their higher rates of cardiovascular disease [43]. The observed ethnic differentials may result not only from income or education but rather culture-specific food selection and intake patterns ranging from food purchasing, sources and preparation, to food combinations. A well-designed prospective study that would measure plasma L-ascorbic acid concentration in subjects and address the above-mentioned ethnic differentials could possibly clarify the impact of vitamin C intake and supplementation on cancer risk.

Vitamin C deficiency in cancer patients

There are numerous data indicating that patients with cancer suffer from L-ascorbic acid deficiency [44]. This phenomenon is caused by a lack of adequate oral intake (anorexia, nausea, vomiting, disorders of the upper gastrointestinal tract resulting

from growing cancer or its local treatment, diet restrictions), decreased bioavailability (intestinal disorders and mucositis resulting from chemotherapy and radiotherapy) and increased tissue utilization [7]. Cancer coexists with chronic inflammation and oxidative stress [45]. Both cause increased consumption of antioxidants, among others L-ascorbic acid. Cancer tissue metabolism, cancer negative impact on patient general metabolism, and prevalence of catabolism resulting from anticancer treatment also accelerate vitamin utilization [46]. Klimant *et al.* reviewed numerous studies showing that L-ascorbic acid blood levels in patients with advanced cancer are significantly lower as compared to earlier stages and healthy subjects. The authors reported vitamin C blood levels of 0.11–1.0 mg/dL (0.00625–0.05678 mM) for patients with different types and stages of cancer, and more pronounced L-ascorbic acid deficiency in the case of advanced disease [7].

Mayland *et al.* revealed that plasma L-ascorbic acid deficiency was found in 15 (30%) of 50 recruited patients with advanced cancer [47]. Low dietary intake, low albumin, high platelet count, high CRP level, and shorter survival were significantly associated with low plasma vitamin C levels (<11 µmol/L).

Mikrova *et al.* measured baseline blood levels of L-ascorbic acid, cancer markers, and C-reactive protein (CRP) in patients with different types of cancer [48]. Following intravenous treatment with L-ascorbic acid, the authors noticed a negative correlation between postinfusional blood level and baseline concentrations of cancer markers and CRP reflecting the stage of the disease and intensity of inflammation. Some studies indicate that systemic anti-cancer treatment also has a negative impact on the L-ascorbic acid level in patient tissues [49]. It was observed that administration of cisplatin, fluorouracil, nilotinib, and interleukin-2 can significantly lower L-ascorbic acid concentration and even result in scurvy-like symptoms. Cytotoxic drugs can generate off-target oxidative stress, which may contribute to depletion of L-ascorbic acid. The onset of scurvy-like symptoms is a common event in many cancer patients, especially in advanced stages, and may not be connected with chemotherapy [44]. Patients with vitamin C deficiency complain about fatigue, myalgia, and arthralgia. They also suffer from nonspecific anemia. There are data showing that discontinuation of chemotherapy may lead to an increase in L-ascorbic acid level [50].

Based on these data it may be concluded that L-ascorbic acid administered intravenously could potentially be used to reduce cancer-related or chemotherapy-induced symptoms and to support health. There are numerous reports on beneficial impacts of therapy with high doses of intravenously administered L-ascorbic acid, which leads to a decrease in inflammatory markers and improvement of symptoms. The same data also indicate that at higher concentrations vitamin

C can reveal cytotoxic potential and inhibit arachidonic acid-derived prostaglandins and, finally, malignant cell proliferation.

Anticancer properties of vitamin C in preclinical studies

Due to its involvement in numerous biological processes, depletion of L-ascorbic acid might be a factor accelerating carcinogenesis and metastasis formation. Cell growth can also be restrained by the transcription factor NF-κB activated by vitamin C [7]. Oxidation or degradation products of L-ascorbic acid (L-dehydroascorbic acid, 2,3-diketogulonic acid, and 5-methyl 1-3, 4-dehydroxytetrone) exhibit antitumor activity [5]. Although at physiological levels vitamin C acts as an antioxidant, its potential therapeutic effectiveness at high doses appears to be linked to pro-oxidant effects that ultimately promote cancer cell death. It may induce cytotoxicity mediated by oxidation generated by hydrogen peroxide. Secondary reactive oxygen species can injure cytoplasmic membranes of cancer cells, which lack adequate levels of catalase activity, as well as escalating cell cycle arrest and p53 upregulation, decreasing ATP levels, impairing mitochondrial function, suppressing antioxidant gene expression, and causing cell death by apoptosis [13]. These phenomena are observed in cell cultures when L-ascorbic acid is present in the medium at a concentration of 1 mM or above [4]. Studies indicate that the cytotoxic L-ascorbic plasma concentration is near or above 400 mg/dL (22.7 mM). To achieve such a blood level of vitamin C, appropriate intravenous administration of at least 60 g is necessary (as discussed in point 7 of this paper).

Yun *et al.* studied the mechanism by which L-ascorbic acid kills *KRAS* and *BRAF* mutant colorectal cancer cells [12]. They revealed that the cytotoxic effect is produced by increased uptake of the oxidized form of vitamin C via overexpressed GLUT1. The role of the transporter was discussed in section 1 of this paper. L-dehydroascorbic acid is reduced to vitamin C, depleting glutathione and causing oxidative stress. Accumulating active oxygen species inactivate glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and cause an energetic crisis and cell death in highly glycolytic *KRAS* or *BRAF* mutant cells. Moreover, the depletion of NAD⁺ due to PARP activation following L-ascorbic treatment further diminishes the GAPDH activity. This phenomenon does not occur in *KRAS* and *BRAF* wild-type cells.

It has also been observed that an addition of vitamin K3 (menadione) to L-ascorbic acid (L-ascorbic acid:K3 ratio of 100:1) produces a synergistic antitumor activity [51]. Administration of such a mixture may cause a new form of

cell death (autoschizis) different from apoptosis, oncosis, or necrosis [41]. Autoschizis is characterized by profound membrane damage, perturbations of the cytoskeleton, and progressive loss of organelle-free cytoplasm through a series of self-excisions that ultimately kill cells [52].

Combination of vitamins C and K3 in a ratio of 100:1 (Apatone) exhibited a synergistic inhibition of growth of different human tumor cell lines (prostate cancer, ovarian cancer, bladder cancer) and induced cell death at concentrations that were from 10 to 50 times lower than required for each of the vitamins individually. The drug was evaluated in a phase I/IIa study [53]. The treatment proved to be effective in inhibiting biochemical progression in prostate cancer patients. However, the therapy was inconvenient for patients. They had to take Apatone orally (500 mg ascorbate and 5mg VK3 as bisulfite), two capsules upon waking up, then one capsule every two hours for six doses followed by two capsules at bedtime, for a total of ten capsules per day for 12 weeks. It could be the reason why the combination was not studied in further trials.

As mentioned before, severe depletion of L-ascorbic acid, even in generally healthy individuals, causes broad tissue disintegration with decomposition of intercellular ground substance as well as interepithelial and interendothelial cement and rupture of collagen bundles. Similar stromal changes are identified in the vicinity of invading neoplastic cells and may contribute to formation of metastases and invasion of cancer cells. There is a theory that stromal resistance may be a physical line of defense against cancer by enclosing neoplastic cells with a compact fibrous tissue, and that this can be improved with L-ascorbic acid treatment [14]. Moreover, vitamin C exhibits an inhibitory effect on hyaluronidases and matrix metalloproteinases, which are also engaged in metastasis formation.

The involvement of vitamin C in humoral immunocompetence and cell-mediated immunity makes it a potential player in anticancer immune response [54]. Magri et al. investigated the concept using the immune competent mouse model of breast, colorectal, melanoma, and pancreatic tumors. They revealed that a high dose of L-ascorbic acid modulated infiltration of the tumor microenvironment by immune cells and delayed cancer growth in a T cell-dependent manner. Vitamin C intensified the cytotoxic activity of adoptively transferred CD8 T cells and exhibited an additive effect with immune checkpoint inhibitor. These promising findings provide a rationale for clinical trials combining immunotherapy with high doses of L-ascorbic acid. Moreover, according to Dan Xi's review, vitamin C can enhance the proliferation and maturation of T cells as well as the proliferation of natural killer (NK) cells. The effect on their immune activity is unknown [17]. There is also no conclusion as to the impact L-ascorbic acid exerts on Tregs.

Neutrophil extracellular traps (NETs) are networks of extracellular neutrophil DNA fibers that can bind tumor cells and may be involved in metastasis formation [55]. Rayes and coworkers demonstrated that circulating NET levels are elevated in advanced esophageal, gastric, and lung cancer patients as compared to healthy controls. Based on pre-clinical murine models of lung and colon cancer, the authors proved that NETs are engaged in regulation of disease progression and that inhibition of NETosis negatively affects spontaneous metastases to the lungs and liver. On the other hand, several studies reported potential reduction of the formation of NETs thanks to IV L-ascorbic acid treatment in patients with sepsis [17]. Along with vitamin C deficiency occurring in neutrophils in the tumor microenvironment, it gives us grounds to assume that intravenous vitamin C infusions could potentially reduce the formation and enhance the clearance of NETs in cancer patients. It is also worth analyzing whether intravenous vitamin C could exert an effect on the number of infiltrating and circulating neutrophils, as it has been proved that Neutrophil to Lymphocyte Ratio (NLR) in the tumor microenvironment and in peripheral blood may have a prognostic value in many different cancers and may predict response to immunotherapy [17, 43, 56].

Another potential therapeutic mechanism of intravenous L-ascorbic acid is its involvement in epigenomic remodeling by enhancing the activity of Jumonji-C domain-containing histone demethylases (JHDMs) [57]. L-ascorbic acid is also a cofactor improving the catalytic activity of ten-eleven translocation (TET) demethylases family especially in vitamin C deficient and/or TET2 mutated tumor cells. As TET2 can suppress production of IL-6, L-ascorbic acid is expected to reduce IL-6—presumed suppression of antitumor immune response [58]. Preclinical studies demonstrated that vitamin C may increase the generation of 5-hydroxymethylcytosine in cultured cells, probably via hydroxylation of 5-methylcytosine by TETs [59]. On the other hand, 5-hydroxymethylcytosine level was substantially decreased in almost all the investigated types of cancer. There are data indicating that physiological concentration of L-ascorbic acid in human serum (10-100 μ M) seems to be adequate for stable level of 5-hydroxymethylcytosine, which is necessary for epigenetic function of the cell [58]. Shenoy et al. [60] demonstrated that reduced 5-hydroxymethylcytosine is associated with decreased survival in clear cell renal cell carcinoma and provides a preclinical rationale for exploring the therapeutic potential of high-dose L-ascorbic acid in such patients. A randomized phase II trial of intravenous L-ascorbic acid combined with pazopanib for metastatic and unresectable clear cell renal cell carcinoma is currently recruiting patients (January 2021, <https://clinicaltrials.gov/ct2/show/NCT03334409>). Shenoy et al. [42] also revealed

that L-ascorbic acid has the potential to modify TET function in lymphoma and enhance chemosensitivity.

A putative role of vitamin C in lymphoma treatment was investigated by Luchtel *et al.* as well. The authors identified a link between genome-wide demethylation and enhanced expression of endogenous retroviral elements in lymphoma cells provoked by L-ascorbic acid treatment and cytotoxic activity of CD8⁺ T cells [61]. The authors also showed that vitamin C increased 5-hydroxymethylcytosine levels of CD8⁺ T cells in lymphoma cultures and hypothesized that it may be an optimal demethylating agent for combination with anti-PD1 therapy as it has also been shown to enhance the function of immune cells such as natural killer (NK) cells, macrophages, and dendritic cells.

The inhibitory effect of L-ascorbic acid on metastases can also be produced by inhibition of HIF [6, 17]. Most solid tumors contain hypoxic regions as a result of limited blood supply to rapidly growing tissue. Main mediators of the hypoxic response are transcription factors, HIF-1 and HIF-2, which upregulate the expression of numerous genes engaged in angiogenesis, glucose uptake, anaerobic metabolism, and cell motility. Intracellular L-ascorbic acid stimulates HIF-1 degradation under normoxic or mildly hypoxic conditions and inhibits the transcriptional activity of HIF-1 and HIF-2.

Impact of L-ascorbic acid on effects of chemotherapy in preclinical studies

Preclinical studies evaluating the impact of L-ascorbic acid on effects of chemotherapy were conducted before 2000. More recent studies are usually focused on vitamin C effect on signaling pathways and metabolic processes engaged in carcinogenesis or targeted treatment, as it was summarized in part 5.

There are data indicating that, when applied at low/physiological doses, L-ascorbic acid may decrease chemotherapy outcomes due to its antioxidant properties (detoxification of reactive oxygen species and reactive nitrogen species) [6]. Its co-administration can potentially reduce toxicity of several chemotherapeutics to normal tissue through suppression of a concurrent oxidative damage in healthy cells. However, studies on pancreatic, liver, prostate and ovarian cancer, sarcoma and malignant mesothelioma models showed that combining high-dose L-ascorbic acid with an anticancer drug can inhibit tumor growth. Vitamin C may directly interact with a redox-active cytotoxic drugs. For example, special attention has been devoted to such interactions with bleomycin. Chelation of iron ions plays a role in the mechanism of action of bleomycin. It has a pseudoenzyme activity, which in the presence of oxygen generates superoxide and hydroxyl radicals that in turn

cleave DNA. When analyzing 2D cultures of human cancer cells and 3D tumor spheroids it was found that restoration of physiological levels of vitamin C in cells increased their killing by bleomycin. Cellular vitamin C enhanced the ability of bleomycin by production of DSBs [62]. Buettner *et al.* also demonstrated that L-ascorbic acid intensified bleomycin-induced DNA damage. However, when it was added to bleomycin prior to exposure to DNA, a redox-inactive drug was produced in a reaction that generated the ascorbyl radical [63].

Prasad *et al.*, in an *in vitro* model of human melanoma, showed that the growth-inhibitory effect of cisplatin, dacarbazine, tamoxifen, and recombinant interferon- α 2b was enhanced by L-ascorbic acid alone, a mixture of three vitamins, that is, beta-carotene, d-alpha-tocopheryl succinate, and 13-cis-retinoic acid, and a mixture of these four vitamins that contained 50 μ g/mL of vitamin C [64].

Chiang and colleagues demonstrated on a human lung cancer line with acquired resistance to vincristine and a unique cross-resistance to other drugs (colchicine, vinblastine, actinomycin, cisplatin, and doxorubicin) that the vincristine resistance was partially reversed by L-ascorbic acid [65]. The drug uptake was enhanced and the mechanism of resistance reversal differed from the P-glycoprotein-mediated multi-drug resistance mechanism. Kurbacher and colleagues published another paper confirming augmentation of chemotherapy effect by L-ascorbic acid in human breast carcinoma cell lines [66]. The authors revealed that combinations of vitamin C with cisplatin and paclitaxel were partly synergistic or partly additive or sub-additive, whereas a consistent synergism was found between L-ascorbic acid and doxorubicin. As regards cisplatin, Reddy *et al.* studied its combination with L-ascorbic acid in the cervical cancer model [67]. They revealed that cisplatin and vitamin C produced the maximum additive effect *in vitro* when the vitamin was used before cisplatin. The authors also demonstrated that the combination of high doses of vitamin C with cisplatin could decrease the effect of chemotherapy, whereas a low dose of the vitamin could have a significant additive effect, particularly when cisplatin was used at a low dose.

In a more recent study, Bhat *et al.* [68] revealed that L-ascorbic acid was able to cause oxidative DNA breakage in normal cells at a concentration of 100-200 μ M and Cu(I) is an intermediate in the DNA cleavage reaction. The authors postulated that in some circumstances a prooxidant action by antioxidants was possible and that even vitamin C plasma concentration of around 200 μ M would be sufficient to cause pharmacological tumor cell death, especially in the case of elevated copper levels.

Chen *et al.* [69] tested a single pharmacologic dose of L-ascorbic acid in mice bearing glioblastoma xenografts and showed that it produced sustained ascorbate radical and

hydrogen peroxide formation selectively within interstitial fluids of tumors, although not in the blood. The authors proved that L-ascorbic acid at pharmacologic concentrations was a pro-oxidant generating hydrogen-peroxide-dependent cytotoxicity toward cancer cells *in vivo* without adversely affecting normal cells.

Moreover Schoenfeld et al. [70] demonstrated that alterations in cancer cell mitochondrial oxidative metabolism resulting in increased levels of O_2^- and H_2O_2 can disrupt intracellular iron metabolism, thereby selectively sensitizing non-small-cell lung cancer and glioblastoma cells to L-ascorbic acid through pro-oxidant chemistry involving redox-active labile iron and H_2O_2 . It was subsequently confirmed in an early clinical trial, which was mentioned in part 9.

Preclinical studies of L-ascorbic acid in cancer models have significant limitations. Cultured cells which are used for biochemical and molecular studies are characterized by limited ability to represent a multicellular context of tissues [6]. Another concern is the concentration of the physiologically relevant nutrients (like L-ascorbic acid) in growth media, which usually significantly differs from *in vivo* conditions. Moreover, animal models also feature some drawbacks regarding investigation of L-ascorbic acid impact on cancer. Most animals produce vitamin C on their own, so dietary intake does not influence blood levels considerably. Absorption of vitamin C in the animal gastrointestinal tract may also be disturbed and such an experiment requires monitoring of vitamin levels in animal serum or tissues [4]. Carr et al. nicely summarized potential interactions between vitamin C and chemotherapeutic agents [71].

Figure 1b diagrammatically displays the impact of L-ascorbic acid on cancer cells and host metabolism in cancer patients.

Administration of pharmacological/high doses of vitamin C

The effect of L-ascorbic acid treatment in cancer patients (as in patients with other conditions) depends on its mode of administration, plasma concentration, and capacity for uptake by tumor cells [14]. Pharmacokinetic studies comparing oral vs. intravenous administration of high doses of vitamin C revealed pronounced differences in its plasma levels. If given orally at a dose of 1.25 g, less than 50% is absorbed and a maximum peak plasma concentration of about 0.1 mM is achieved. This falls back to a baseline of about 0.07 mM after four hours. The same dose administered intravenously results in a peak plasma level of 1mM. That difference in plasma levels of L-ascorbic acid after oral and parenteral administration comes out of homeostatic down-

regulation of its sodium-dependent transporter 1 (SVCT1) on intestinal epithelial cells in the presence of elevated levels of vitamin C [8]. Intravenous administration of doses above 0.5 g allows bypassing of the tight control of L-ascorbic acid absorption after oral intake. Parenteral infusion of vitamin C at high/pharmacological doses can result in peak plasma concentrations that are several hundred times higher than those possible with maximal oral doses until renal excretion brings back homeostasis. It is estimated that the saturation point of renal tubular reabsorption of L-ascorbic acid is 0.07 mM and the clearance time of intravenous vitamin C ranges from 30 minutes to 2 hours. Moreover, cancer patients excrete less L-ascorbic acid from all intake sources than healthy subjects [7].

A discovery of pharmacokinetic properties of L-ascorbic acid in healthy adults renewed interest in its possible value in cancer treatment.

Studies evaluating intravenous L-ascorbic acid alone and in combination with chemotherapy in cancer patients showed that it was well tolerated with an adverse event rate of about 1% [7]. Reported side effects of intravenous vitamin C administration include, among others, nausea, mild headache, dizziness, dry mouth, hyperhidrosis, and weakness. These symptoms result mainly from the osmotic load [8]. In order to prevent their occurrence, patients are advised to have intensive oral hydration before and during infusion of vitamin C.

When intravenous treatment with L-ascorbic acid is planned, caution should be exercised in patients with iron and copper storage diseases, renal failure, history of kidney stones or oxaluria, and pregnancy or lactation. Doses higher than 75 g (and blood concentrations exceeding 10 mM) might be contraindicated also in patients with history of dehydration, severe pulmonary edema, anuria, or heart failure. However, there is a report on a clinical benefit of high dose intravenous L-ascorbic acid in anemia of chronic diseases in patients with chronic kidney disease [7]. Oxalic acid is a major end product of vitamin C oxidation and it has the potential to crystallize as calcium oxalate in the urinary tract. In normal conditions, intestinal absorption of vitamin C is limited and the risk of increased oxalic acid excretion and stone formation is minimal. However, there are case reports of oxalate nephropathy in patients with renal dysfunction and increased urinary oxalic acid excretion receiving parenteral nutrition solutions containing 200 to 500 mg/d of L-ascorbic acid. However, prospective trials have not supported the association of vitamin C intake and kidney stones. Robitaille et al. measured urinary oxalic acid excretion in 16 patients with advanced cancer participating in a phase I clinical trial of intravenous L-ascorbic acid [72]. Using adequate methodology, they showed that less than 0.5% of a very high intravenous dose of vitamin C was recovered as urinary oxalic

acid in people with normal renal function. Authors concluded that in the case of a relatively slow natural history of oxalate stone accumulation, exposure to intravenous L-ascorbic acid up to three times per week would not create a severe risk of this complication in patients without other risk factors. However, caution is required in patients with a high risk of oxalate stones which may be further increased by high-dose vitamin C.

Caution is also recommended in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency because of the risk of hemolytic events. G6PD level should be tested 8–12 weeks after potential blood transfusion. Its normal level is 4.6–13.5 U/g of hemoglobin [7]. Notably, various doses of vitamin C and different duration of infusions can produce diverse serum levels with pro- or anti-oxidant properties. Increase of vitamin C intravenous dose without extending the infusion time can produce plasma levels creating a differential gradient and driving the vitamin into the tissue space, theoretically stimulating pro-oxidant behavior. Klimant *et al.* postulate that there are no absolute low and high doses of intravenous vitamin C, but rather “low-dose behavior” and “high-dose behavior,” which results from the dose, the infusion time, and the disease status of an individual [7]. They suppose that “high-dose behavior” can give pro-oxidative effects. The authors also provide some recommendations regarding intravenous infusion of L-ascorbic acid in supportive care. That indication for vitamin C treatment seems to be quite well documented [73, 74, 75, 76]. They advise administration of vitamin C before possible chemotherapy, followed by a 30- to 60-minute break, or 12–72 hours after chemotherapy depending on the half-life and clearance of the chemotherapy. They justify this approach with theoretical concerns about the administration of intravenous antioxidant treatment during curative chemotherapy and any possible reduction in treatment efficacy. The recommended approach allows for clearance of L-ascorbic acid before administration of cytotoxic drugs. The authors suggest that L-ascorbic acid in an intravenous dose of 5–25 g infused over a period of 30 to 120 minutes is a safe option for cancer-affected adults of any sex and body mass, as it results in reduction of inflammation, optimal supplementation of the body’s antioxidant stores, and possibly improvement of quality of life.

Stephenson *et al.* investigated safety, tolerability, and pharmacokinetics of high-dose intravenous L-ascorbic acid (up to 110 g/m²) as a monotherapy in patients with advanced solid tumors [77]. The minerals (calcium, magnesium, and potassium) in solution containing vitamin C were in chloride forms because clinical experience with intravenous vitamin C has shown a chloride shift, which results in hypochloremia that requires compensation. The initial concerns about possible formation of oxalate stones (large amounts of magnesium had been added to inhibit it) and chelation of

serum calcium with L-ascorbic acid and potential tremors due to hypocalcemia (high levels of calcium were added as prevention) diminished with growing clinical experience. Therefore, the concentrations of calcium and magnesium in the mixture were reduced to the same levels as concentrations in plasma. The infusion solution should be administered via a central venous access catheter at a rate of 1 g/min because it tended to be hyperosmolar (1,200 mOsm/L) and difficult to tolerate when administered in a peripheral vein. The infusion bag of L-ascorbic acid should be protected from light to prevent photo-oxidation [77].

Clinical studies of L-ascorbic acid in cancer treatment

During the last five decades, growing body of evidence concerning pharmacokinetics of L-ascorbic acid in humans, its roles as biological cofactor, and potential anticancer properties resulted in early phase clinical trials dedicated to cancer patients [17]. There are also numerous case reports and observational studies regarding this population of patients. The most important studies are summarized in Table 1.

E. Cameron and A. Campbell were pioneers in this field. In 1974, they communicated the clinical response of fifty consecutive advanced cancer patients to the continuous administration of large intravenous doses of L-ascorbic acid. They concluded that it was of value in palliative care of terminal cancer [6, 7, 79, 80, 96]. Several subsequent trials investigating high doses of intravenous L-ascorbic acid in cancer patients shown that it allows increased quality of life; improves physical, mental, and emotional functions; and induces less frequent adverse effects of standard anticancer treatment, including fatigue, nausea, vomiting, and appetite loss [73, 74, 75, 76]. For example, it was reported that breast cancer and ovarian cancer patients experienced less severe chemotherapy-toxicity if such therapy was combined with intravenous L-ascorbic acid treatment [74, 81]. Moreover, Stephenson *et al.* showed that in pretreated patients with advanced solid tumors, intravenous administration of L-ascorbic acid was well tolerated, even at doses up to 1.5 g/kg of body weight or 70–80 g/m² of body surface and up to 110 g/m² with stabilization of disease in 3/17 patients [77]. Furthermore, the same studies showed that administration of vitamin C could exhibit analgesic properties in some diseases accompanied by acute or chronic pain [97]. There are also data indicating that cancer-related pain may be reduced with high dose L-ascorbic acid, which contributes to a patient’s enhanced quality of life [73, 83, 92]. Among many postulated mechanisms of vitamin C analgesic properties, there is

Table 1. Studies evaluating the role of vitamin C in cancer treatment carried out in years 1978-2018

Main author and year of publication	Study design	Results
Cameron 1978 [78]	Observational with matched controls; IV and oral vitamin C vs. no vitamin C; 1,100 patients (100 treated with vitamin C), advanced malignancies; concurrent treatment not defined; primary outcome: survival	Mean survival from the date of commencing best supportive care - 298 days in a vitamin C-treated arm vs. 38 days in controls (p= 0.01)
Murata 1982 [79]	Observational without a control group; IV and oral vitamin C at a high dose ($\geq 5\text{g/d}$) vs. IV and oral vitamin C at a low dose or no vitamin C; 99 patients with terminal cancer at the Fukuoka Torikai Hospital and 31 patients at the Kamioka Kozan Hospital; concurrent treatment not defined; primary outcome: survival	Cohort 1: the average time of survival after the date of designation as terminal was 43 days for 44 low-vitamin C patients and 246 days for 55 high-vitamin C patients; Cohort 2: the average survival time was 48 days for 19 control patients and 115 days for six high-vitamin C patients; no confidence intervals or any result of a test of significance reported, the administration of large doses of vitamin C seemed to improve the quality of life
Harris 2013 [80]	Observational, retrospectively assessed; vitamin C oral intake divided by quartiles, 3405 women diagnosed with breast cancer at all stages; concurrent therapy not described; primary outcome: survival regarding an vitamin C intake	The highest vs. lowest vitamin C intake prior to breast cancer diagnosis associated with benefit in mortality, HR 0.75 (0.57-0.99); no association between vitamin C intake following breast cancer diagnosis and mortality
Takahashi 2012 [75]	Observational, oral and IV vitamin C; 63 patients with newly diagnosed cancer, stage not defined, 34 patients undergoing concurrent chemotherapy; primary outcome: quality of life assessed using the QOL questionnaire developed by the European Organization of Research and Treatment of Cancer (EORTC) - EORTC-QLQ C30	The global health/QOL score significantly improved from 44.6 ± 27.8 to 53.2 ± 26.5 ($p < 0.05$) at week 2 and to 61.4 ± 24.3 ($p < 0.01$) at week 4; a significant increase in physical, role, emotional, cognitive, and social functioning at week 4 ($p < 0.05$), observed a significant relief of fatigue, pain, insomnia, constipation, and financial difficulties, no adverse events reported
Yeom 2007 [76]	Observational; oral and IV vitamin C; 39 patients with metastatic cancer, no concurrent treatment; primary outcome: quality of life	An improvement in global health score from 36 ± 18 to 55 ± 16 after 1 week of vitamin C treatment
Vollbracht 2011 [81]	Observational retrospective study; IV vitamin C plus standard surgery, chemotherapy, radiation, and hormonal treatment (n=53) vs. standard therapy alone (n=72); 125 breast cancer patients (stages IIa-IIIb); primary outcome: quality of life (symptom intensity score, trial specific)	An improvement in quality of life in a vitamin C group based on intensity of complaints score ($p = 0.013$); appetite, nausea, fatigue, depression, and sleep disorders during and after adjuvant therapy significantly improved in the vitamin C group; no validated 'intensity of complaints' scale used; no survival outcomes reported
Gunes-Baiyr 2015 [73]	Retrospective; IV vitamin C 2.5 g/d (n=15) vs. chemotherapy (n=15) vs. control (n=9); 39 patients with bone metastases, all the patients were radiotherapy-resistant; primary outcome: pain intensity, performance status, and survival	An increase in performance status in four patients of a vitamin C group and in one patient of chemotherapy group, in control group a decrease was observed; a median 50% reduction in pain in the vitamin C group; median survival 10 months in the patients receiving vitamin C vs. 2 months in the chemotherapy and control groups
Lv 2018 [13]	Retrospective; IV vitamin C 2 g for four or more days after initial hepatectomy (n=339) vs. control (n=274); 613 HCC patients who received curative liver resection; primary outcome: survival	5-year disease-free survival (DFS) for patients who received IV vitamin C was 24% vs. 15% for controls ($p < 0.001$); Median DFS for IV vitamin C users 25.2 vs. 18 mo. for control ($p < 0.001$); multivariate analysis demonstrated that IV vitamin C was an independent factor for improved DFS (adjusted HR = 0.622, 95% CI 0.487 to 0.795, $p < 0.001$)
Hoffer 2008 [82]	Phase I; oral and IV vitamin C dose-escalating + oral multivitamin; 24 patients with advanced malignancies; primary outcome: dose finding; secondary - toxic effects, antitumor effects, quality of life using the Functional Assessment of Cancer Therapy — General (FACT-G) questionnaire, effects of different IV doses on the plasma vitamin C profile	1.5 g/kg vitamin C infused >90-120 min three times weekly is free of risk and important side-effects when simple precautions are taken; response rate: 0%; adverse events and toxicity were minimal at all dose levels; patients in the 0.4 g/kg cohort experienced a significant deterioration in physical function over the course of the study, however, there was no deterioration in physical function among the patients in the higher dose cohorts; no changes in the social, emotional or functional parameters of quality of life in any cohort
Stephenson 2013 [77]	phase I; multivitamin and eicosapentaenoic acid (EPA); IV vitamin C 30-110 g/m ² ; 17 patients with advanced solid tumors; primary outcome: pharmacokinetics, safety and tolerability	Toxicity: grade 3-4 hyponatremia, hyperkalemia; in three patients stable disease, in 13 progressive disease observed; recommended dose of vitamin C 70-80 g/m ²
Kiziltan 2014 [83]	Pilot study; IV vitamin C; 11 patients with bone metastases who were unresponsive to standard cancer treatments; primary outcome: efficacy, toxicity	The mean reduction in pain was 55%; median survival: ten months; toxicity: 40% grade 1 gastrointestinal toxicity and 30% urinary toxicity

Main author and year of publication	Study design	Results
Polireddy 2017 [84]	Phase I/IIa; IV vitamin C + gemcitabine; 14 pancreatic cancer patients; primary outcome: safety and pharmacokinetic interaction	Twelve of 14 patients completed phase I pharmacokinetic evaluation and entered Phase IIa; median survival: 15.1 months; median progression-free survival: 3 months; significant adverse events: none were deemed related to IV vitamin C; adverse events attributable to IV vitamin C were grade 1 nausea and thirst
Welsh 2013 [85]	Phase I/II; IV vitamin C + gemcitabine; 11 advanced pancreatic adenocarcinomas; primary outcome: toxicity	Data only for nine patients who completed the study; two patients progressed and were excluded from the analysis; mean survival: 13 +/- 2 months; time to progression: 26 +/- 7 weeks; no dose limiting adverse effects
Pinkerton 2017 [86]	An open-label pilot study; oral vitamin C in conjunction with opioids and standard adjuvant therapy; 34 patients with chronic pain secondary to cancer and/or its treatment; primary outcome: efficacy in the management of chronic cancer pain	Seven patients failed to complete the trial; 17 evaluable patients; the median daily opioid consumption was 360 mg oral morphine equivalents on the days prior to vitamin C and 390 mg when administered with vitamin C
Schoenfeld 2018 [70]	Phase I; following maximum safe surgical resection or biopsy (if unresectable), subjects received radiation (daily), temozolomide (daily), and IV vitamin C (three times a week) for approximately seven weeks, for each subsequent subject, vitamin C doses were escalated during the radiation phase (15-125 g) with 20 mM target plasma concentration; 13 glioblastoma multiforme patients; primary outcome: safety and tolerability	Following completion of the radiation phase, all subjects were dose escalated to achieve ≥ 20 mM plasma vitamin C in combination with temozolomide for approximately 28 weeks; the desired mean therapeutic blood level was achieved in all the subjects; vitamin C was safe and well tolerated with minimal grade 3 and 4 toxicities; two subjects were excluded from a long-term analysis due to non-adherence to the protocol therapy because of unrelated comorbidities; all the subjects maintained their performance status throughout the treatment; median progression-free survival: 9.4 months; median survival: 18.2 months.; in 73% of patients MGMT promoter methylation was absent and median survival in this group was 23.0 months
Monti 2012 [8]	Phase I/II; gemcitabine and erlotinib and IV vitamin C with dose escalation design; 14 patients with stage IV pancreatic adenocarcinoma; primary outcome: safety	Two patients who decided to withdraw from the study, and three patients who died from rapid disease progression were excluded from the survival analysis; mean OS: 182 days, (standard deviation 155 days); mean PFS: 89 days; response rate: 0%; stable disease rate: 50%; multiple toxicities, all grades, however, not related to vitamin C
Berenson 2009 [87]	Phase I/II; bortezomib, melphalan and oral AA; 35 newly diagnosed multiple myeloma patients; primary outcome: response rate and safety	Median survival: not reached at time of the publication (range 2-231 months); median progression-free survival: 13 months (range 2-221 months); response rate 74%; toxicity related to bortezomib and melphalan, not to vitamin C; grade 2 side effects predominantly hematological; the author stated that results were inferior to those obtained with other agents
Riordan 2005 [88]	Phase I/II; IV vitamin C vs. no; 24 patients with advanced malignancies; primary outcome: safety	Response rate: one stable disease (4%); toxicity: four grade 3-4 events (renal calculi, hypokalemia)
Tareen 2008 [53]	Phase I/IIa study; vitamin C 5000 mg: K350 mg (Apatone) oral; 17 patients with 2 successive increases in PSA after unsuccessful standard local therapy; primary outcome: safety and efficacy	PSAV decreased and PSADT increased in 13 of 17 patients ($p \leq 0.05$); there were no dose-limiting adverse effects
Nielsen 2017 [89]	Phase II; IV vitamin C weekly infusions (week 1.: 5 g; week 2.: 30 g, and week 3-12. 60 g) with ongoing androgen-deprivation therapy with castration-level of testosterone; 23 patients with chemotherapy-naïve, metastatic castration-resistant prostate cancer; primary outcome: the efficacy (reduction of prostate-specific antigen – PSA level) and safety; secondary endpoints: changes in health-related quality of life, biomarkers of bone metabolism, inflammation and bone scans	Twenty patients completed the study; no patient achieved a 50% PSA reduction; instead, a median increase in PSA of 17 $\mu\text{g/L}$; among the secondary endpoints, no signs of disease remission were observed; 53 adverse events, including 11 serious adverse events; three events were directly related to vitamin C (fluid load)
Zhao 2018 [90]	Randomized controlled trial, A-DCAG (IV vitamin C plus DCAG: decitabine, cytarabine, aclarubicin) [n = 39]] vs. DCAG (n = 34); 73 elderly patients with acute myeloid leukemia (AML); primary outcome: efficacy	Patients who received A-DCAG presented a higher complete remission (CR) rate than those who received DCAG (79.9% vs. 44.1%; $p = 0.004$) after first induction; median survival was longer in a A-DCAG group as compared to a DCAG group (15.3 months vs. 9.3 months, $p = 0.039$); similar toxicity

Main author and year of publication	Study design	Results
Goel 1999 [91]	Randomized controlled trial; oral vitamin C plus CMF chemotherapy vs. chemotherapy; 30 patients with stage IIIB/IV breast cancer; primary outcome: response in breast	60% (vitamin C+ chemotherapy) vs. 33.3% (chemotherapy) measured by Vernier calipers, criteria for response not defined; change in tumor size by Vernier calipers 3.53 cm (vitamin C) vs. 1.93 cm (placebo); results of tests for significance not reported
Ma 2014 [74]	Randomized controlled trial; IV vitamin C plus chemotherapy (carboplatin and paclitaxel) vs. chemotherapy alone; 27 patients with stage III-IV ovarian cancer; primary outcome: toxicity	Statistically significant decrease in grade 1-2 toxicities in vitamin C group based on Common Terminology Criteria for Adverse Events, v. 3; 8.75-month increase in median progression-free survival in the AA arm (p value not reported); a trend to improved OS in the vitamin C group based on Kaplan-Meier analysis; no numerical data reported
Yeon 2016 [92]	Randomized controlled trial; IV vitamin C 50 mg/kg vs. placebo immediately after induction of anesthesia; 100 patients undergoing laparoscopic colectomy; primary outcome: effect of vitamin C on opiate consumption assessed at 2, 6, and 24 h after the completion of surgery	Ninety-seven patients included in the analysis; patients who received vitamin C had higher plasma concentrations of vitamin C at the end of surgery, significantly lower morphine consumption 2 h after the surgery, and significantly lower pain scores at rest during first 24 h after surgery; there were no significant differences between the groups in side effects, fatigue score, or pain score on coughing
Creagan 1979 [93]	Randomized controlled trial; oral vitamin C vs. placebo; 123 patients with advanced cancer; primary outcome: survival	No significant difference in survival, and no difference in symptoms of disease
Moertel 1985 [94]	Randomized controlled trial; oral vitamin C vs. placebo; 100 patients with advanced colorectal cancer; primary outcome: survival	Median survival: 2.9 months (vitamin C) vs. 4.1 months (placebo); no significant difference in quality of life
Zemskov 2000 [95]	Randomized controlled trial; IV and oral vitamin C plus Ukrain (anticancer drug based on the extract of the plant <i>Chelidonium majus</i> L.) vs. IV and oral vitamin C alone; 42 patients with advanced pancreatic adenocarcinoma, two patients had surgery; primary outcome: survival	Median survival 17 months in Ukrain plus vitamin C arm vs. 6.97 months in a vitamin C arm (p=0.001); response rate: 14.3% in a Ukrain group, vs. 0%, respectively; criteria for response not defined

one suggesting that it is a cofactor for the biosynthesis of amidated opioid peptides.

Despite such optimistic results of observational and uncontrolled studies as well as numerous case reports, unfortunately no randomized controlled trials reported any statistically significant improvements in overall or progression-free survival with L-ascorbic acid, as compared to control arm [89, 95]. The data regarding reduced toxicity of chemotherapy with L-ascorbic acid are inconclusive.

Conclusions and final remarks

Vitamin C plays a role in numerous biological reactions, and its deficiency may potentially contribute to different diseases, including cancer, among others. Although there are some data that patients with decreased L-ascorbic acid intake may have an increased risk of head and neck cancer, as well as lung, gastric, pancreatic, cervical, rectal, or breast cancer, we lack clinical evidence to support a preventive effect of antioxidant supplements (L-ascorbic acid, among others) against cancer. Therefore, reasonable consumption of L-ascorbic acid (from dietary sources) required for general health and optimum well-being is indicated. The supplementation of vitamin C as cancer prevention is not supported by clinical evidence. In Poland, the recommended dietary allowance (RDA) of

vitamin C is 75 mg/d for adult women and 90 mg/d for men (approximately 1 mg per 1 kg of body mass), for pregnant and breastfeeding women these values are 85 mg/d and 120 mg/d, respectively [98]. According to other guidelines, optimum plasma concentration, which is similar to the level of saturation (70 mM), requires a daily intake of about 200 mg [99].

A growing body of evidence also indicates the role of L-ascorbic acid in cancer treatment, especially as a part of supportive care. However, considering the above-mentioned possible interactions between L-ascorbic acid and chemotherapy, there are still questions about optimal dosing and timing of administration. Questions about pharmacokinetics of opiates combined with L-ascorbic acid should also be answered.

New preclinical data indicates a role of L-ascorbic acid in modulation of immune response, and its involvement in epigenomic remodeling may contribute to its new potential clinical applications in cancer patients, especially if combined with immunotherapy. Also, administration of high-dose L-ascorbic acid in patients with glioma who undergo radiation and temozolomide therapy seems interesting in view of the vitamin's good ability to penetrate the brain tissue and achieve the potential target, which is alteration in cancer cell mitochondrial oxidative metabolism [14, 70]. L-ascorbic acid is relatively inexpensive and low-toxic, and

intravenous infusions have a positive impact on quality of life and presumably on life prolongation (data from retrospective studies) in patients with advanced cancer. Considering all these facts, it seems reasonable to further investigate L-ascorbic acid in high-quality, placebo-controlled trials, especially as supportive treatment or in combination with targeted therapy, with properly selected end-points.

Authors' Contribution

S.D.S.: research concept and design, acquisition of data, analysis and interpretation of data, designing the figures, drafting the article or revising it critically for important intellectual content, writing the manuscript, literature review, final proofreading and approval of the version for publication;
P.P.: research concept and design, supervising the project, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final proofreading and approval of the version for publication.

Conflict of Interest

The authors have no potential conflicts of interest to declare.

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