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REVIEW PAPER

Ascorbic acid in cancer management – time for a second look

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ABSTRACT

Introduction and aim. Over the past decades, the hypotheses that ascorbic acid (AA) can play a role as an anti-neoplastic therapy have generated many conflicting reports. Despite the controversies, mounting evidence has shown that AA has the potential to play a role as an anti-neoplastic agent. Recent studies have unraveled its pharmacokinetics and various mechanism of action on cancer cells. This has spawned different preclinical studies with reports of good activities against various cancers.

Material and methods. A review of the literature regarding ascorbic acid in the management of cancer was performed using the PubMed database. The research was limited to abstracts and available full-text articles.

Analysis of the literature. Clinical trials have also demonstrated its safety and tolerability across different dosages. AA has been noted as a multitargeting agent that acts as a pro-oxidative cytotoxic agent, anti-cancer epigenetic regulator and immune modulator. AA has also been shown act synergistically with standard chemotherapy regimens in different cancers. Despite its potentials, phase III clinical trials are seriously lacking. The recent phase III VITALITY study shows that AA may play a role as an adjunct targeted therapy for ras-mutated cancers. Therefore, there is need to for more standardized clinical trials to help identify cancer subtypes and AA combination regimens that can show the most benefits. In this review, the pleiotropic mechanism of action of AA was explored as well as various preclinical and clinical studies in cancer therapy. In addition, recommendations were also made for effective strategies towards an AA and standard cancer regimens in treatment as well as future directions. Ascorbic acid has been shown to induce cell death in various cancer types through different mechanisms of action. Several clinical trials and case reports have shown its efficacy in combination chemotherapy, and the pharmacological route of action can be either intravenous or oral. However, it can impair the actions of some drugs when given in combination. Also, dosage should be determined for maximal pharmacologic action.

Conclusion. Ascorbic acid has the potential to provide safe and cost-effective antineoplastic treatment option especially in combination therapy. Its potential needs to be further investigated through clinical trials.

Keywords. ascorbic acid, cancer, clinical trials

Introduction

Ascorbic acid (vitamin C, AA) is a six- carbon lactone that is synthesized from glucose and primarily acts as an electron donor at the physiological state. It is a known pleiotropic molecule that serves various functions in the human body ranging from collagen hydroxylation, metabolism of folic acid, tyrosine and tryptophan, synthesis of carnitine and catecholamines and neutralizing free radicals as well as protection of DNA damage (Fig. 1).[1]

Apart from its physiological roles, AA has been muted as a potential anticancer agent. Several experimental studies have shown that AA at pharmacological doses has some clinical effects on various types of cancer.[2-4] Since the 1950s, AA has been proposed as a

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cancer agent, however, in 1974 works carried out by the Scottish surgeon Ewan Cameron and his colleague Allan Campbell using high dose AA both intravenously and orally as a cancer treatment showed the drug as tolerable and safe, and with a complete remission in one case later reported.[5,6] Cameron and Pauling later on showed that AA significantly prolonged survival in terminal cancer patients.[7] Their work was met by criticisms which were largely procedural; which led to a second investigation by the men and this time the new study showed that patients on high dose AA had a mean survival time of about 300 days longer than the untreated controls.[8] However, two Mayo Clinic randomized, placebo-controlled prospective trials later disproved the efficacy of AA in cancer patients.[9-10] However, the Mayo clinic trial was faulted on two grounds; only patients with colorectal cancer were used in the trial which is not representative enough of the various cancer types. More importantly, unlike Cameron's subjects that received both intravenous and oral AA, subjects in the Mayo Clinic trials received only oral AA. The importance of the route of administration determines the degree of bioavailability of AA.[11]

Subsequently, a gradually increasing number of preclinical studies and some clinical studies have shown the efficacy of AA especially when given intravenously. Some experimental works have shown that high dose AA induce growth arrest in tumor cells both in vitro and ex vivo.[11-13] Padayatty et al. in their work showed that peak plasma AA concentrations were higher after administration of intravenous doses than after administration of oral doses, and this difference increased with the dose. When AA was given at a dose of 1.25 g orally, it produced a peak plasma concentration of 134.8±20.6 μmol/L, while the IV administration produced a peak plasma concentration of 885±201.2 μmol/L.[14] The IV administration is thus seen as the pharmacologic basis of action.

Aim

Material and methods A review of the literature regarding ascorbic acid in the management of cancer was performed using the PubMed database. The research was limited to abstracts and available full-text articles.

Analysis of the literature

Ascorbic acid homeostasis

Oral administration of AA is tightly controlled via intestinal absorption, accumulation and distribution in tissues, utilization and recycling, and renal excretion and reabsorption.[15-16] These processes are ensured through different ways including passive diffusion, facilitated diffusion, active transport and recycling.[17-19] After the ingestion of AA, it is absorbed into the bloodstream. The intestinal absorption of AA has been observed to be reduced with increased intake up to a certain dose; this is due to a decrease in the expression of the sodium AA transporter sodium-dependent vitamin C transporters (SVCT). AA is known to be taken up primarily into cells via SVCT 1 and 2, on the other hand, its oxidized form, dehydroascorbate (DHA), is taken up via the facilitated diffusion transporters glucose transporters (GLUTs).[15,19-20] The AA transporter SVCT1 which is expressed primarily in intestine, liver and kidney is known to mediate the renal reabsorption of AA. Mice lacking the SVCT1 gene have been reported to increase AA fractional excretion up to 18-fold with hepatic portal AA accumulation nearly terminated; however, the intestinal absorption was mildly affected.[21] SVCT2 on the other hand, which is expressed in almost all cells, contributes to the accumulation of AA in most tissues.[22] SVCT2 deficiency has been linked to perinatal mortality in mice, and elevated risk of spontaneous preterm births in humans.[22,23] This is probably as a result of poor AA accumulation.

Oral intake of AA is tightly controlled as a result of these regulatory processes. Whereas, the intravenous administration of AA have been shown to achieve a 70-fold higher plasma levels than even the highest oral tolerable dose.[14] Interestingly, at these higher plasma concentrations via intravenous administration AA is able to kill cancer cells making it an emerging potential anticancer therapy.

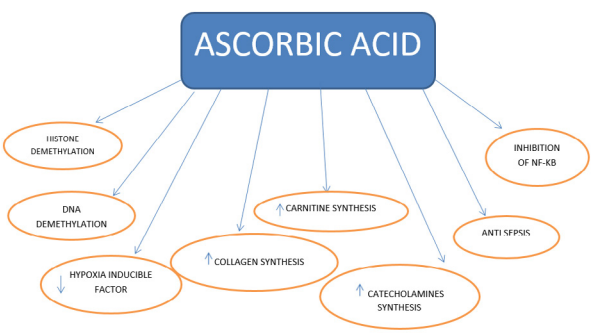

Fig. 1. Some of the different functions of AA

Antineoplastic activity of AA

AA has been shown to be an antioxidant through the suppression of free radicals generation, as well as attenuation of oxidative damages caused by the free radicals.[24-25] Examples of its antioxidant activity can be found in the prevention of low-density lipoprotein oxidation, and reduction in amyloid plaques in the nervous system.[26-28] AA supplementation is also reported to increase the levels of glutathione and thiols and negatively affects the levels of oxidative stress markers malondialdehyde, and nitrites.[29] AA also acts an anti-inflammatory in different pathological state, including cancer, sepsis, stroke etc.[30-33]

Antioxidant

Importantly, as part of its antioxidative properties AA is the reprogramming of the epigenome through the enhancement of the catalytic activity of the Jumonji-C domain-containing histone demethylases (JHDMs) and the ten-eleven translocation (TET) family of DNA hydroxylases which drive histone and DNA demethylation in somatic cells; thus AA can modulate embryonic stem cell function, enhance reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) and hinder the aberrant self-renewal of hematopoietic stem cells (HSCs).[34-37] In addition, AA also regulates the DNA methyl transferases (DNMT), the hypoxia inducible transcription factor alpha prolyl hydroxylases D (PHD) and the histone alpha/beta hydrolase (ABH).[38-40] This aforementioned activity of AA i.e. regulation of gene expression, is associated with its antioxidant function and has important role in cancer treatment. While antioxidant therapies have been promoted as potential antineoplastic agents, caution should be applied in the indiscriminate prescription of antioxidant supplements. Reactive oxygen species (ROS) are the primary targets of antioxidants, and are known to aid tumour growth.[41-42] This is not so in all cases; an excess of ROS can destroy cancer cells which is a mechanism used by some chemotherapy and radiotherapy.[42-44] However, based on the notion that ROS aid tumor growth, anti-oxidant therapies have been muted as likely antineoplastic therapies. This may not be so true, as some evidence shows that antioxidant activities are also deployed in tumourigenesis and metastasis.[45-48] Besides, Yasueda et al. in their systematic review were of the opinion that it is difficult to determine whether antioxidant supplements affect treatment outcomes.[59] This position was also muted by Watson.[50] Therefore, pro-oxidant therapies may be more amenable to some cancers.

Pro-oxidant

High-dose AA may just be a potential candidate for pro-oxidant therapy. Different mechanisms have been proposed for the pharmacologic action of AA on cancer cells. One of them is the pro-oxidant action of AA on cancer cells. According to the pro-oxidant theory, AA pro-oxidant activity occurs at higher concentrations in the presence of iron. Iron is reduced by AA to Fe^{2+} in the presence of oxygen which leads to the formation of hydrogen peroxide (H_2O_2) and reactive oxygen species. The H_2O_2 further reacts with Fe^{2+} to generate a highly reactive hydroxyl radical. However, in normal tissues H_2O_2 generated is quickly neutralized by the appropriate enzymes e.g. catalase. These enzymes in tumour cells can be defective which lead to the persistence of H_2O_2 and subsequent cell damage.[51-52] Another mechanism of cancer cell death involves DHA. DHA is an oxidized form of AA that is transported into the cell via the facilitated glucose transporters GLUTs.[53] Cancer cells take in DHA and it is reduced back to AA.[54] This intracellular reduction back to AA cause a depletion of glutathione in the cell, which consequently lead to an increase in ROS, oxidative stress, energy crisis and cell death.[55-56] In another study, DHA is also reported to reacts with homocysteine thiolactone (cancer cells have high levels of homocysteine thiolactone) converting it to the toxic compound, 3-mercaptopropionaldehyde and kills the cell.[57]

AA and intracellular labile iron

AA is also reported to target iron (Fe) for various physiological processes (Fig. 2). Iron is an important nutrient that plays various roles in the body such as oxygen homeostasis, cellular metabolism and DNA synthesis.[58-60] Iron is normally transported across the cells in the form of a transferrin (Tf)-Fe^{3+} complex through the cell surface receptor transferrin receptor 1 (TfR1) and then it is moved in via endocytosis. Fe^{3+} (ferric ion) is reduced to Fe^{2+} (ferrous ion) after acidification by the the endosomal six transmembrane epithelial antigen of the prostate 3 (STEAP3). Fe^{2+} is then transferred across the endosomal membrane by divalent metal transporter 1 (DMT1). The Fe^{2+} forms part of the labile iron pool (LIP) – a pool of chelatable and redox-active iron, which is serves as a crossroad of cell iron metabolism. LIP is known to promote formation of reactive oxygen species (ROS) via the Fenton chemistry.[60,61] Fe^{2+} can be oxidized back to Fe^{3+} via the Haber-Weiss reaction with the formation of hydroxyl radicals ($OH^{\bullet}$ and OH^{-}).[62] The presence of AA can prevent Fe^{2+} oxidation by reductive accelerating the Fe^{3+}/Fe^{2+} cycles, and lowering of the redox potential of Fe^{3+}/Fe^{2+} through chelating effect, which leads to enhanced ROS production with more lipid, protein, and DNA oxidation. With these, AA is seen to play a role in cell death through ferroptosis – a novel form of regulated cell death mediated by iron-dependent lipid peroxidation.[63] Ferroptosis have been muted as a promising approach in cancer therapy.[64,65] AA is thought to also play a role in the regulation of iron metabolism by the stimulation of ferritin synthesis, inhibition of lysosomal ferritin degradation and reduction of cellular iron efflux.[66]

Alteration in iron metabolism is generally associated with tumourigenesis which usually involves increased intercellular iron import and reduced iron export. For example, breast cancer patients have significantly higher levels of iron than normal controls.[67,68] Thus, cancers with high levels of iron might be more susceptible to AA through increased production of free radicals via LIP. In a research reported by Xia et al., high dose AA, in the presence of iron, leads to the formation of highly ROS resulting in cell death of multiple myeloma cells.[69]

AA and hypoxia

The iron containing alpha-ketoglutarate-dependent hydroxylases (α-KGDD) are another substrate for AA.

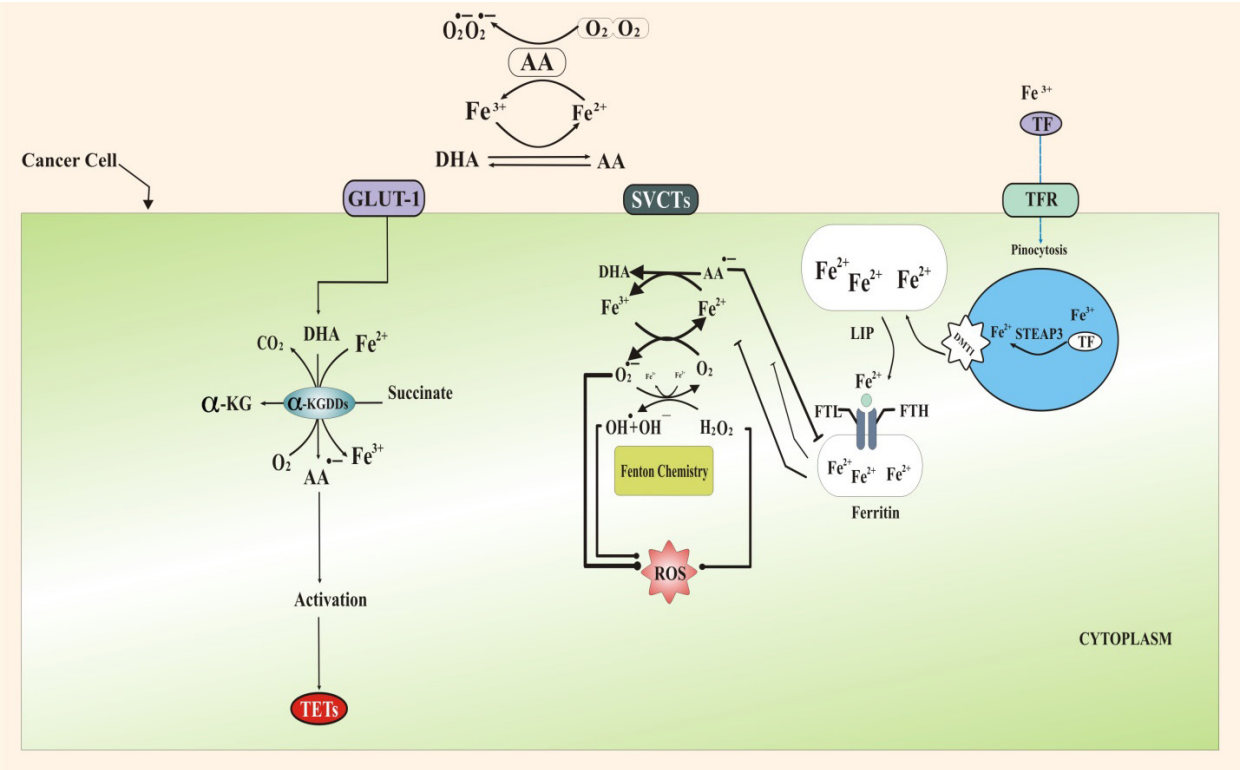

Fig. 2. AA is involved in the imbalance of LIP through the formation of the TF-Fe^{3+} complex which is later acidified by STEAP3, with Fe^{3+} reduced to Fe^{2+}. The accumulated Fe^{2+} generates LIP which interacts with AA and oxygen to produce DHA and Fe^{3+} which later produce ROS through the Fenton reaction. The ROS can lead to cancer cell death through the process of ferroptosis and apoptosis. AA can also act as a co-factor for TET enzymes leading to some epigenetic modifications

α-KGDD catalyze oxidation reactions by incorporating a single oxygen atom from molecular oxygen (O_2) into their substrates. Examples include asparagine hydroxylase and proline hydroxylase. They regulate the activity of hypoxia inducible factor 1α (HIF-1α). HIF-1α is a hetero-dimeric transcription factor that is regulated by hypoxia. They can also be activated by non-hypoxic pathways.[70,71] Prolyl hydroxylase domain (PHD) proteins are known to hydroxylate proline residues on HIF-1α in normoxic situations. After the hydroxylation, the von Hippel Lindau tumor suppressor protein binds to the prolyl-hydroxylated HIF1-α, activating an E3-ubiquitin ligase which targets it for proteasome degradation. On the other hand, asparagine hydroxylase hydroxylates HIF-1α at asparagine residues on the C-terminus, preventing the recruitment of p300/CBP co-activators, thereby making HIF-1α inactive. The reverse is the case in a hypoxic situation, with PHD and arginine hydroxylase suppressed leading to the translocation of HIF-1α into the nucleus where it dimerizes with HIF1-β (also known as aryl hydrocarbon nuclear receptor translocator (ARNT)). The HIF-1α- HIF1-β complex then binds to hypoxia response elements leading to the upregulation of a number of genes. HIF-1α is known to be up-regulated in various cancers.[72,73] HIF-1α also portends a poor prognosis in some cancers.[74,75] Due to its role in cancers, HIF-1α is seen as a viable target for cancer therapy. Most inhibitors work in an indirect mode such as bortezomib and camptothecin and its derivatives.[76] Recently, the FDA approved belzutifan, the first-in-class HIF inhibitor for adult patients with von Hippel-Lindau (VHL) disease – associated tumours.[77] AA has been shown to suppress HIF1-α-dependent cancer growth.[78,79] It does this primarily through the increase in activity of arginine and PHD hydroxylases, therefore decreasing HIF1-α action and cancer growth.[80] Research conducted on cancer patients show an association between AA, HIF-1 activation, and cancer growth. Tissues from these patients showed that cancers with the most potent HIF1 function were those lacking AA in its tumour microenvironment, and patients with higher levels of AA had better outcomes.[81,82] Thus, AA can prevent cancer development through HIF. More researches will be needed to validate this.

AA and NF-κB

Chronic inflammation is one of the hallmarks of carcinogenesis.[83] In the role of inflammation in cancer, one of the key players is the transcription factor nuclear factor-kappaB (NF-κB) which is responsible for signaling processes in immunity, inflammation, cell proliferation and survival. NF-κB consist of five structurally related members which are NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB and c-Rel, and together they are involved in the activa-

tion of certain target genes by binding to the κB enhancer – a specific DNA element, as hetero- or homo-dimers.[84] In the quiescent cell, NF-κB typically binds to its inhibitors IκB (IκBα, IκBβ, IκBγ, IκBε, Bcl-3, p100, and p105) in the cytoplasm making it transcriptionally inert. However, upon stimulation IκB is phosphorylated by IκB kinase (IKK), which cause the release of NF-κB and its translocation into the nucleus where it induce the upregulation and transcription of target genes involved in pro-inflammatory response including cyclooxygenase-2 (COX-2) and inducible nitric oxide (NO) synthase. Interestingly, NF-κB is involved in many aspects of cancer development and survival, and it is a target for many small molecules. NF-κB is also known to be regulated by redox control mechanisms; thus, its actions can be adjusted based on ROS concentration.[85-89] This activity is bidirectional.[90] It has been reported that low concentrations of ROS activate the IKK/IκB/NF-κB signal pathway, whereas high concentrations of those inhibit the activation.[91] Du et al. showed that AA via its oxidative product DHA, could inhibit NF-κB through massive ROS generation mediated by intracellular glutathione and copper ions.[91] Studies have shown that AA can inhibit NF-κB through other mechanisms. AA has been reported to block the activation of NF-κB by Tumor necrosis factor-alpha (TNF alpha) through the activation of p38 Mitogen-Activated Protein Kinase (MAPK), and also DHA directly inhibited IKKB and IKK alpha enzymatic activity in vitro independent of p38-MAPK, whereas AA did not.[92-94] These anti- NF-κB activities were ROS- independent. However, it is not all gloom. AA has been shown to be involved in the epigenomic and transcriptomic remodeling of monocyte-derived dendritic cells (DC). The P65 subunit of NF-κB is known to interact with TET2 protein of the epigenome in DCs, and during such interactions, AA triggers an extensive demethylation at NF-kB/p65 binding sites together with concordant upregulation of antigen-presentation immune response-related genes during DC maturation.[95] In addition, AA causes an increase in the production of tumor necrosis factor-beta (TNFβ) in DCs through NF-κB; the selective inhibition of NF-κB I DCs is reported to block maturation and proliferation of T cells.[96,97] These studies show that AA could play a role in some cancers with high NF-κB activity.

AA and epigenetic regulation

The TET proteins are a part of the α-KGDD – a family of non-heme proteins, that are involved in the hydroxylation of 5-methylcytosine (5mC) residues to 5-hydroxymethylcytosine (5hmC) leading to demethylation of DNA residues and activation of certain gene transcriptions (Fig. 3). This epigenetic regulation is an important hallmark in many malignancies. In solid tumours, TET2 mutation is uncommon.[98] However, it is frequently mutated in haematological cancers.[99] DNA hypermethylation as a result of TET2 mutation is associated increased risk of MDS progression, and poor prognosis in AML.[100,101] AA is an epigenetic regulator. It acts as a cofactor for optimal TET activities by reducing Fe^{3+} and Fe^{2+} which results in active DNA demethylation. In addition, mutations in the enzymes isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) lead to metabolic alterations and the formation of 2-hydroxyglutarate (2-HG), an oncometabolite that can inhibit the activity of α-KGDD such as ten-eleven translocation (TET) enzymes reducing 5hmC, boosting DNA methylation and leading to an inhibition of normal cell differentiation. In an AML model, AA was shown to induce an IDH-dependent reduction in cell proliferation and an increase in expression of genes involved in leukocyte differentiation.[102] Also, the hypomethylating agents, azacitidine and decitabine are cytidine analogs that cause DNA demethylation as a result of DNA methyltransferase-1 (DNMT-1) inhibition; including being active in TET2 mutated haematologic malignancies.[103-105] Gerecke et al. showed that addition of AA to hypomethylating agents caused the increased expression of the tumour suppressor p21 (CDKN1A), and induction of apoptosis.[106] A clinical trial (NCT02877277) involving MDS and AML patients showed that AA supplementation in patients on hypomethylating agents induced epigenetic changes.[107] While TET2 is known to have a pleiotropic role in hematopoiesis, it is equally known to promote leukemogenic predisposition especially in haematopoietic stem cells through its regulation of access of some key transcription factors to enhancers of target genes.[108] AA has been found to strengthen the DNA demethylation by TET2 in haematopoietic stem cells, thereby suppressing leukemogenesis and aiding lineage differentiation.[109-112] Thus, AA can play a role in the prevention and management of haematological malignancies. A very recent clinical trial published by Taira et al. showed that AA can also boost DNA demethylation in TET2 germline mutation carriers strengthening the case for AA supplementation in haematological malignancies.[113]

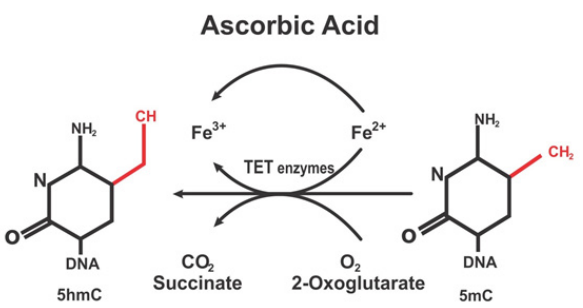

Fig. 3. The TET enzymes are involved in the catalysis of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) with AA as a co-factor through the transfer of an electron from Fe^{2+} to Fe^{3+}, and eventual activation of TET enzymes

AA and cancer immunotherapy

One of the modes of cancer resistance is immune evasion; this is a mechanism through which cancer cells camouflage themselves from immune cells of the body preventing their detection and destruction. These mechanisms are important for cancer progression and metastasis.[114] Furthermore, most subset of immune cells are fingered in cancer biology.[115] One way cancer cells impair immunity is through the high expression of immune checkpoint proteins such as programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). The immune checkpoint proteins naturally serve as costimulatory/coinhibitory molecules that provide the necessary checkpoint regulating T cells and other APCs' interactions as well as balancing immune homeostasis.[116] However, cancer cells express them as a way to suppress the immune system and escape from immune surveillance.[117] Anti-checkpoint inhibitors were therefore developed for the management of cancers that express them. Ipilimumab, an anti-CTLA-4 became the first checkpoint inhibitor to be approved in 2011 for the management of metastatic melanoma.[118] Others like nivolumab and pembrolizumab later followed. Currently, anti-PD-1 (nivolumab and pembrolizumab), anti-PD-L1 (Atezolizumab, avelumab, and duravulumab), and anti-CTLA-4 (ipilimumab, tremelimumab), are the FDA-approved checkpoint inhibitors for the management of a broad range of cancers. However, increased toxicities and treatment failures were some of the challenges encountered in the clinics.[119,120] Thus, reducing the toxicity of checkpoint inhibitors, while increasing their efficacy is an unmet clinical need. One way of achieving this, is the use of intravenous AA in pharmacological doses.[121]

AA has been shown to enhance cancer immunotherapies in both in vivo and in vitro studies. In a very recent study published, Burkard et al. were able to verify the efficacy of high dose AA to kill melanoma cells both in vitro and in vivo; and how it also exert their effect either alone or in combination with the anti-mouse checkpoint inhibitor antibody synergistically.[122] In another study, Luchtel et al. showed that the combination of AA with a checkpoint inhibitor could have significant activity in cancer treatment. Their evaluation was the combination of high dose AA with a checkpoint inhibitor in a lymphoma mouse model. Their findings were that AA i) increases immunogenicity of lymphoma cells; ii) enhances intratumoral infiltration of CD8+ T cells and macrophages; and iii) synergizes with anti-PD1 checkpoint inhibition in a syngeneic lymphoma mouse model via marked activation of cytotoxic cells (cytotoxic T cells and NK cells) and antigen presenting cells.[123] In their own study, Magri et al. showed that a combination of AA with anti-PD1 or anti-CTLA4 in mice resulted in a significant reduction of tumour volume in breast, pancreatic and colonic cancers.[124] Just like the Luchtel's group, they also reported an increase in T cell infiltration of the tumour microenvironment.[124] In the same vein, a recent study by Peng et al. showed that a combination of AA and PD-L1 inhibitor in murine renal cell carcinoma caused increased intramural infiltration of T cells as well as the expression of chemokines and cytokines.[125]

AA has equally been shown to contribute to immune defense especially the innate and adaptive immune system. The immune cells especially macrophages and neutrophils are known to accumulate AA which they use to protect themselves against reactive oxygen species and enhance chemotaxis and phagocytosis.[126-128] Neutrophil extracellular traps (NETs) – net-like structures composed of DNA-histone complexes and proteins released by activated neutrophils and are involved in many disease state, have been identified in neutrophil loss, tumour progression and metastasis as well as the promotion of T cell exhaustion.[129-131] A study by Mohammed et al. showed that in vitro administration of AA to human neutrophils caused a decrease in phorbol ester-induced NETosis.[132] In another study, AA incubated with human neutrophils from septic patients with reduced chemotactic and phagocytic activities showed a decrease in spontaneous NETosis formation and an improvement in neutrophil function.[133] Like the phagocytes, B, T lymphocytes and NK cells are also known to accumulate high levels of AA.[134,135] It is not certain why, though it is believed for antioxidant protection. AA has been linked to the development and maturation, proliferation and differentiation of the lymphocytes.[136-139] These activities of AA on immune cells have been muted to benefit chimeric antigen receptor (CAR) cells to enhance their efficacy against cancer cells.[140,141] Kouakanou et al. showed that addition of AA during CD19-CAR T cell production enforces a stem cell memory–like phenotype and enhance anti-tumour function.[140] Huijskens et al. showed this was also applicable to NK cell therapy.[142] Recently, γδ T cells have been shown to be potential effector cells in cell-based cancer immunotherapy.[143] This has attracted a fast track designation by the FDA for the allogeneic γδ T cells for the treatment of relapsed or refractory B-cell non-Hodgkin lymphoma.[144-146] AA has been shown to enhance the proliferation and effector functions of human γδ T cells.[147] This ability to expand γδ T cells as well as enhance their effector function have led to the first adoptive transfer of allogeneic γδ T cells expanded in vitro in the presence of vitamin C into patients with solid cancers, and which showed increased survival in those patients.[148] AA has effect on cancer immunotherapy, but more clinical studies would need to be carried out to determine just how useful they are.

AA and clinical studies

AA is used by many complementary and alternative cancer therapists, and for the past decade or more there

have been a steady rise in the evaluation of AA as an antineoplastic therapy. Some of these trials evaluated its use as a monotherapy or as combination therapy with standard chemotherapies. While the pre-clinical studies have shown potentials, the results of the clinical studies have been mixed. Zasowska-Nowak et al. in their review reported that from various studies done AA was ineffective in human studies conducted in advanced-stage cancer patients.[150] This was a bit different from a systematic review reported by Mohseni et al. who found that high dose AA with chemotherapy resulted in an increase in overall survival (OS); however, as a monotherapy, AA was tolerable and safe but without any objective tumour response.[151] Nielsen et al. in their study on prostate cancer patients reported no signs of disease remission.[152] This may have been so, because the dosage and frequency administered in this study was much lower compared to those reported by Stephenson et al. and Hoffer et al., who reported stable disease in 3 and 2 patients respectively, but also without objective tumour response.[153,154] While AA monotherapy may be less efficacious, however, a handful of random case reports have shown that AA monotherapy could possibly be efficacious in the treatment of some cancers.[155-157] This was best exemplified in a series of case reports by Raymond et al. among some patients treated in Singapore.[158] The case of an AML patient in palliative care who achieved complete remission for 2.5 years while on high dose AA also shows the efficacy of AA monotherapy.[159] Currently, some AA monotherapy studies are ongoing (NCT03613727) (NCT03682029).[160,161]

Clinical studies of AA in combination therapy

Some studies of AA as a combination therapy with chemotherapies have also been reported. However, they were carried out with only a limited number of patients, and no double blind randomzed trials. Some of the combinations have shown promise in some cancers. In a phase 1 clinical study of patients with metastatic gastric and metastatic colorectal cancers given IV AA at 1.5 g/kg once daily with mFOLFOX6 or FOLFIRI with or without bevacizumab in a 14 day cycle, Wang et al. reported a favourable safety profile and an objective response rate (ORR) of 58.3% with a disease control rate of 95.8%.[162] This result spurred a randomized, open label, multicenter phase 3 study of IV AA + FOLFOX ± bevacizumab (experimental group) vs. FOLFOX ± bevacizumab alone (control group). However, in the recently published result the experimental group failed to show superior progression free survival (PFS) compared to the control group [median PFS, 8.6 vs. 8.3 months; HR, 0.86; 95% confidence interval (CI), 0.70–1.05; P = 0.1]; but patients with RAS mutation had significantly longer PFS (median PFS, 9.2 vs. 7.8 months; HR, 0.67; 95% CI, 0.50–0.91; p=0.01) with AA added to chemotherapy.[163] Some other small successes have been recorded in AA plus chemotherapy combination therapy. A phase 1 clinical trial (PACMAN) of IV AA and gemcitabine in patients with metastatic or unresectable pancreatic adenocarcinoma showed good clinical safety and tolerability, and has now been escalated to a randomized phase 2 trial and it's currently ongoing (PACMAN 2.1) (NCT02905578).[164,165] Polireddy et al. also reported a phase I/IIa clinical trial (NCT01364805) in pancreatic cancer patients using IV AA and gemcitabine. The study showed an OS of 15.1 months with one of the participant showing significant tumour response.[166] Their study showed that AA has a multi-targeting mechanism of action on pancreatic cancer cells including ATP depletion and increased α-tubulin acetylation. AA has been shown to have good activity against RAS-mutated cancers, and since more than 90% of pancreatic cancers harbor the RAS mutation, AA could possibly have some activity against pancreatic cancers. In addition, AA has been shown to alter cancer metabolism.[163,167-170] A preclinical study of AA plus buformin on AML cell lines shows that AA inhibits glucose metabolism through interfering with hexokinase 1/2 and GLUT1 functions in hematopoietic cells as well as depletes ATP production.[171] Currently, a phase 2 clinical trial of AA in combination with metformin in some solid tumours is ongoing (NCT04033107).[172]

AA and haematological malignancies in clinical studies

A number of clinical studies have also been done with haematological malignancies (Table 1). Some of the studies involved the use of arsenic trioxide. In a clinical study (ChiCTR1800018811) by Qian et al. which aimed at comparing the efficacy and tolerability of an arsenic trioxide/bortezomib/ascorbic acid/dexamethasone (ABCD) regimen with efficacy and tolerability of a bortezomib/dexamethasone (BD) regimen in patients with newly diagnosed myeloma, the ABCD regimen showed a greater response rates (above VGPR) than the BD regimen. It also showed significantly improved PFS and better tolerability with lower bone marrow suppression especially in patients with low or standard risk disease.[173] In two similar phase I/II study by Berenson et al., arsenic trioxide/bortezomib/ascorbic acid (ABC) combination therapy in patients with relapsed/refractory multiple myeloma was well tolerated and showed an objective response rate (ORR) of 27% in the heavily pretreated study population; and also the melphalan, arsenic trioxide (ATO) and ascorbic acid (AA) (MAC) combination therapy for patients with multiple myeloma showed an ORR of 48% with good safety and tolerability.[174,175]

Similarly, in patients managed for acute promyelocytic leukemia (APL) given oral arsenic trioxide, all-trans retinoic acid and ascorbic acid (popularly known as the triple

Table 1. Clinical trials using ascorbic acid as anti-neoplastic therapy in hematological malignancies

Trial number	Cancer type	Phase	Study design	Combination	AA dose
ChiCTR1800018811[173]	Multiple myeloma	II	Randomized	Arm 1: AA + chemotherapy (ABD)	1000 mg IV days 1 to 3, 8–10, and 15–17 over 30 mins
				Arm 2: Chemotherapy (BD)	
NA[174]	Multiple myeloma	NA	Single arm	Bortezomib and Arsenic Trioxide	1 g IV on days 1, 4, 8, and 11 of a 21–day cycle for a maximum of 8 cycles
NCT04251754[176]	Acute promyelocytic leukemia	III	Observational	Arsenic Trioxide and All trans retinoic acid	1 g/day for 6 weeks (oral)
NCT03682029[160]	Myeloid malignancies	II	Interventional (randomized)	Placebo	1 g daily for 12 months (oral)
NCT03999723[185]	Myeloid malignancies	II	Interventional (randomized)	Arm 1: AA + Azacitidine	1 g daily (oral)
				Arm 2: placebo + Azacitidine	
ACTRN12621000223831[184]	Myeloid malignancies	II	Non-randomized, open trial	Arm 1: (TET2 mutation) azacitidine	IV 30g on days 1–5, 8–9 or days 1–7
				Arm 2: (RAS +/- TET2 mutation) azacitidine and lenzilumab	
NCT03418038	Lymphoma	II	Interventional (Randomized)	Arm 1: Ascorbic acid + combination chemotherapy	IV on days 1, 3, 5, 8, 10, 12, 15, 17 and 19
				Arm 2: Placebo + combination chemotherapy (rituximab + ifosfamide + carboplatin + etoposide D1–3; rituximab + cisplatin + cytarabine + dexamethasone if MR or SD after 2 courses)	
				Arm 3: Ascorbic acid + combination chemotherapy (ifosfamide + carboplatin + etoposide or cisplatin + cytarabine + dexamethasone or gemcitabine + dexamethasone + cisplatin or gemcitabine + oxaliplatin or oxaliplatin + cytarabine + dexamethasone)	

A regimen), the leukemia-free survival (LFS) and overall survival (OS) rates were 100% at 3 years and 94.1% at 5 years respectively.[176] In a similar study, APL patients who achieved first complete remission (CR) and were placed on triple A maintenance had a 5-year and 10-year rates of relapse-free survival (RFS) of 89% and 85%, and OS of 94% and 87%, respectively.[177] The triple A regimen was also shown to be safe and associated with long term survival in patients. The use of the Triple A regimen in APL as maintenance therapy is still ongoing in another study (NCT04251754).[178-180] AA is also known to induce DNA demethylation at the cellular level. In a clinical study by Zhao et al., 73 elderly AML patients treated with AA plus a combination of decitabine, cytarabine, aclarubicin and granulocyte colony-stimulating factor (DCAG) had a higher CR (79.92% vs. 44.11%; p=0.004) after one cycle of chemotherapy, and a median OS (15.3 months vs. 9.3 months, p=0.039) compared with the DCAG only group.[181] Welch et al., in a clinical phase 1 study of AA, decitabine and arsenic trioxide in patients with MDS and AML observed complete remission with incomplete blood count recovery (CRi) in one patient, and stable disease (SD) in five patients after four cycles of therapy.[182] A suggestion for a phase II trial was muted. TET2 is mutated in many haematological malignancies, and plays a major role in epigenetic modulation alongside prognosis of myeloid neoplasms.[99,101,183] Due to the role AA play as an epigenetic modifier, targeting demethylases with AA in combination therapy has been muted as therapeutic strategy.[34] Currently, the PREACH-M study a phase II trial of the use of AA with azacitidine and lenzilumab in CMML patients (ACTRN12621000223831) is ongoing.[184] Other similar clinical studies using CMML, MDS and AML patients (NCT03682029) (NCT03999723) are also ongoing.[160,185]

Time for a second look?

The jury is out, and after several pre-clinical and clinical studies on the potentials of AA as an antineoplastic agent, a verdict is yet to be reached. The pertinent question still remains, should randomized clinical trials be organized to test for the benefit of AA in cancer? For this author it is an affirmative yes. However, this should be within the confines of a well-designed randomized clinical trial (possibly, double-blinded) and preferably as a combination therapy; the phase (induction or maintenance) should also be determined.

Different issues needs to be resolved for a standardized randomized clinical trial, including dosage and frequency which can be dependent on the route of administration. Padayatty et al. determined that only an I.V administration can produce a pharmacologic dose for anti-tumour activity.[14] However, oral AA has been shown to have some activities.[107] So under which conditions oral AA can be used need also to be determined. In the VITALITY study by Wang et al., AA with chemotherapy was shown to induce a significantly longer PFS in CRC patients with RAS mutation than patients on chemotherapy alone.[163] In the EudraCT 2018-000155-41 clinical trial, Taira et al. showed that AA supplementation reinforces DNA demethylation in TET2 mutation carriers; Das et al. equally reported a complete remission in an AML patient with TET2 mutation on AA.[113,159]

This means clinical trials can be designed for AA as a targeted therapy, though AA is a known multitargeting agent in cancer.[186] This "promiscuity" as a multi-targeting agent can make the development of a biomarker as an indicator of response to AA therapy rather problematic. Clinical markers and response such as tumour size shrinkage, overall survival and improved quality of life may just be more durable measurements in such cases.

AA has also been shown to attack cancer by modulating the immune system.[187] Thus, pharmacological dose of AA can potentially serve as an adjunct anti-neoplastic therapy.[141,147] Clinical trials are required to test the efficacy of AA as a potential adjunct anti-neoplastic therapy. In pre-clinical studies, AA has been shown to have synergism with checkpoint inhibitors.[122,123] AA has also been shown to synergistically potentiate the cytotoxicity of targeted therapies ibrutinib, venetoclax and idelalisib in CLL.[188] Further clinical investigations would be needed to determine the clinical benefits. While AA is known to synergize with many anti-neoplastic drugs, it antagonizes in some cases.[189] Dhahri and Chhabra reported of a case of impaired effect of AA on imatinib in a CML patient.[190] Heaney et al. in their pre-clinical study also reported that AA antagonizes the cytotoxic effect of some commonly used chemotherapeutic drugs including imatinib.[191] This inhibitory effect is also reported with bortezomib. It has been reported that AA directly inactivates bortezomib activity by forming a tight but reversible complex through its vicinal diol group.[192] However, the dose used for this study was not pharmacological and it was in oral form. AA is also muted to be able to inactivate ixazomib because of the boronate moiety. However, in a clinical study reported by Bolaman et al., AA enhanced the cytotoxic effect of carfilzomib-lenalidomide-dexamethasone in relapsed/ refractory myeloma patients who initially did not respond to the treatment; thus AA may have no effect on carfilzomib at pharmacological dose.[193-199] In a phase I-II clinical trial (NCT01050621), Hoffer et al. recommended carrying out trials in higher numbers in order to identify specific clusters of cancer type, chemotherapy regimen and AA combination in which exceptional responses are observed to justify a more focused clinical trial.[196] This position is appropriate. The crosstalk between Cabanillas and his colleagues is a good example that a more focused randomized clinical trial is needed to put AA in a proper position for cancer therapy.[197-199] Further investigations of AA action on proteasome inhibitors will be needed. In general, the pharmacokinetics and pharmacodynamics of AA should be considered in the design of any clinical trial.

Conclusion

AA is reported to be generally low in cancer patients. The relationship between AA and cancer is a subject of intense study. While current reports on the anti-neoplastic activity of AA is mixed, it is known to be well-tolerated and possibly play a role in supportive care in cancer. Unlike recent innovative therapies, AA is more affordable for everyone. The use of AA in combination with standard cancer therapies should be further explored in randomized clinical trials. It is time for a second look.

Declarations

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Author contributions

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Conflicts of interest

The author declare no conflicts of interest.

Data availability

Not applicable.

References

1. Iqbal K, Khan A, Ali Khan Khattak MM. Biological significance of ascorbic acid (Vitamin C) in human health. A review. *Pakistan Journal of Nutrition*. 2004;3(1):5-13. doi: 10.3923/pjn.2004.5.13
2. Roa FJ, Peña E, Gatica M, et al. Therapeutic Use of Vitamin C in Cancer: Physiological Considerations. *Front Pharmacol.* 2020;11:211. doi: 10.3389/fphar.2020.00211
3. Chen P, Stone J, Sullivan G, Drisko JA, Chen Q. Anti-cancer effect of pharmacologic ascorbate and its interaction with supplementary parenteral glutathione in preclinical cancer models. *Free Radic Biol Med.* 2011;51(3):681-687. doi: 10.1016/ j.freeradbiomed.2011.05.031
4. McCormick WJ. Cancer: the preconditioning factor in pathogenesis; a new etiologic approach. *Arch Pediatr.* 1954;71(10):313-322.
5. Cameron E, Campbell A. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact.* 1974;9(4):285-315. doi: 10.1016/0009-2797(74)90019-2
6. Cameron E, Campbell A, Jack T. The orthomolecular treatment of cancer. III. Reticulum cell sarcoma: double complete regression induced by high-dose ascorbic acid therapy. *Chem Biol Interact*. 1975;11(5):387-93. doi: 10.1016/0009-2797(75)90007-1
7. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci USA*. 1976;73(10):3685-3689. doi: 10.1073/pnas.73.10.3685

8. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci USA*. 1978;75(9):4538-4542. doi: 10.1073/pnas.75.9.4538
9. Creagan ET, Moertel CG, O'Fallon JR, et al. Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. *N Engl J Med*. 1979;301:687-690. doi: 10.1056/NEJM197909273011303
10. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. *N Engl J Med*. 1985;312:137-141. doi: 10.1056/NEJM198501173120301
11. Chen Q, Espey MG, Krishna MC, et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci USA*. 2005;102(38):13604-13609. doi: 10.1073/pnas.0506390102
12. Cha J, Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Ascorbate supplementation inhibits growth and metastasis of B16FO melanoma and 4T1 breast cancer cells in vitamin C-deficient mice. *Int J Oncol*. 2013;42(1):55-64. doi: 10.3892/ijo.2012.1712
13. Cha J, Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Ascorbate depletion increases growth and metastasis of melanoma cells in vitamin C deficient mice. *Exp Oncol*. 2011;33(4):226-230.
14. Padayatty SJ, Sun H Wang Y, et al. Vitamin C pharmacokinetics: Implications for oral and intravenous use. *Ann Intern Med*. 2004;140:533-537. doi: 10.7326/0003-4819-140-7-200404060-00010
15. Levine M, Padayatty SJ, Espey MG. Vitamin C: a concentration-function approach yields pharmacology and therapeutic discoveries. *Adv Nutr*. 2011;2(2):78-88. doi: 10.3945/an.110.000109
16. Lindblad M, Tveden-Nyborg P, Lykkesfeldt J. Regulation of vitamin C homeostasis during deficiency. *Nutrients*. 2013;5(8):2860-2879. doi: 10.3390/nu5082860
17. Wilson JX. Regulation of vitamin C transport. *Annu Rev Nutr*. 2005;25:105-125. doi: 10.1146/annurev.nutr.25.050304.092647
18. Przybyło M, Langner M. On the physiological and cellular homeostasis of ascorbate. *Cell Mol Biol Lett*. 2020;25:32. doi: 10.1186/s11658-020-00223-y
19. Tsiaoussis GI, Christaki E and Apidianakis Y. I Can C Clearly Now: How EPEC Inhibits Gut Vitamin C Transport by Dysregulating SVCT. *Dig Dis Sci*. 2021;66:2140-2142. https://doi.org/10.1007/s10620-020-06594-8
20. Tsukaguchi H, Tokui T, Mackenzie B, et al. A family of mammalian Na+-dependent L-ascorbic acid transporters. *Nature*. 1999;399(6731):70-75. doi: 10.1038/19986
21. Corpe CP, Tu H, Eck P et al. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. *J Clin Invest*. 2010;120(4):1069-1083. doi: 10.1172/JCI39191
22. Sotiriou S, Gispert S, Cheng J, et al. Ascorbic-acid transporter Slc23a1 is essential for vitamin C transport into the brain and for perinatal survival. *Nat Med*. 2002;8(5):514-517. doi: 10.1038/0502-514
23. Erichsen HC, Engel SA, Eck PK, et al. Genetic variation in the sodium-dependent vitamin C transporters, SLC23A1, and SLC23A2 and risk for preterm delivery. *Am J Epidemiol*. 2006;163(3):245-254. doi: 10.1093/aje/kwj035
24. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition*. 2002;18(10):872-879. doi: 10.1016/s0899-9007(02)00916-4
25. Monacelli F, Acquarone E, Giannotti C, Borghi R, Nencioni A. Vitamin C. Aging and Alzheimer's Disease. *Nutrients*. 2017;9(7):670. doi: 10.3390/nu9070670
26. Kiokias S, Proestos C, Oreopoulou V. Effect of Natural Food Antioxidants against LDL and DNA Oxidative Changes. *Antioxidants*. 2018;7(10):133. doi: 10.3390/antiox7100133
27. Samsam Shariat SZ, Mostafavi SA, Khakpour F. Antioxidant effects of vitamins C and e on the low-density lipoprotein oxidation mediated by myeloperoxidase. *Iran Biomed J*. 2013;17(1):22-28. doi: 10.6091/ibj.1092.2012
28. Kook SY, Lee KM, Kim Y, et al. High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice. *Cell Death Dis*. 2014;5(2):e1083. doi: 10.1038/cddis.2014.26
29. Waly MI, Al-Attabi Z, Guizani N. Low Nourishment of Vitamin C Induces Glutathione Depletion and Oxidative Stress in Healthy Young Adults. *Prev Nutr Food Sci*. 2015;20(3):198-203. doi: 10.3746/pnf.2015.20.3.198
30. Mikirova N, Casciari J, Rogers A, et al. Effect of high-dose intravenous vitamin C on inflammation in cancer patients. *J Transl Med*. 2012;10:189. doi: 10.1186/ 1479-5876-10-189
31. Muhammad M, Jahangir A, Kassem A, et al. The Role and Efficacy of Vitamin C in Sepsis: A Systematic Review and Meta-Analysis. *Adv Respir Med*. 2022;90(4):281-299. doi: 10.3390/arm90040038
32. Farrag N, Elwakeel L, Abdelhafeez A, Schaalan M. High Dose Vitamin C Improves Inflammatory Markers and Clinical Outcome Of Patients With Acute Respiratory Distress Syndrome. *Archives of Pharmaceutical Sciences Ain Shams University*. 2021;5(2):304-316. doi: 10.21608/aps.2021.94382.1069
33. Sánchez-Moreno C, Dashe JF, Scott T, Thaler D, Folstein MF, Martin A. Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke. *Stroke*. 2004;35(1):163-168. doi: 10.1161/01.STR.0000105391.62306.2E
34. Lee Chong T, Ahearn EL, Cimmino L. Reprogramming the Epigenome With Vitamin C. *Front Cell Dev Biol*. 2019;7:128. doi: 10.3389/fcell.2019.00128

35. Huff TC and Wang G. Rewriting the Script: The Story of Vitamin C and the Epigenome. In: *Handbook of Nutrition, Diet, and Epigenetics*. Patel VB, Preedy VR, ed. Springer;2019;1671-1690. doi: 10.1007/978-3-319-55530-0_46
36. Cimmino L, Neel BG, Aifantis I. Vitamin C in Stem Cell Reprogramming and Cancer. *Trends in Cell Biology*. 2018;28(9):698-708. doi: 10.1016/ j.tcb.2018.04.001.
37. Brabson JP, Leesang T, Mohammad S, Cimmino L. Epigenetic Regulation of Genomic Stability by Vitamin C. *Front Genet*. 2021;12:675780. doi: 10.3389/fgene.2021.675780
38. Venturelli S, Sinnberg TW, Berger A, et al. Epigenetic impacts of ascorbate on human metastatic melanoma cells. *Front Oncol*. 2014;4:227. doi: 10.3389/fonc.2014.00227
39. Osipyants AI, Poloznikov AA, Smirnova NA, et al. L--ascorbic acid: A true substrate for HIF prolyl hydroxylase? *Biochimie*. 2018;147:46-54. doi: 10.1016/j.biochi.2017.12.011
40. Mandl J, Szarka A, Bánhegyi G. Vitamin C: update on physiology and pharmacology. *Br J Pharmacol*. 2009;157(7):1097-110. doi: 10.1111/j.1476-5381.2009.00282.x
41. Kuo CL, Ponneri Babuharisankar A, Lin, YC, et al. Mitochondrial oxidative stress in the tumor microenvironment and cancer immunoescape: foe or friend? *J Biomed Sci* 2022;29:74. doi: 10.1186/s12929-022-00859-2
42. Aggarwal V, Tuli HS, Varol A, et al. Role of Reactive Oxygen Species in Cancer Progression: Molecular Mechanisms and Recent Advancements. *Biomolecules*. 2019;9(11):735. doi: 10.3390/biom9110735
43. Möhler H, Pfirrmann RW, Frei K. Redox-directed cancer therapeutics: Taurolidine and Piperlongumine as broadly effective antineoplastic agents (review). *Int J Oncol*. 2014;45(4):1329-1336. doi: 10.3892/ijo.2014.2566
44. Wondrak GT. Redox-directed cancer therapeutics: molecular mechanisms and opportunities. *Antioxid Redox Signal*. 2009;11(12):3013-3069. doi: 10.1089/ars.2009.2541
45. Harris IS, Treloar AE, Inoue S, et al. Glutathione and thioredoxin antioxidant pathways synergize to drive cancer initiation and progression. *Cancer Cell*. 2015;27(2):211-222. doi: 10.1016/j.ccell.2014.11.019
46. Lignitto L, LeBoeuf SE, Homer H, et al. Nrf2 Activation Promotes Lung Cancer Metastasis by Inhibiting the Degradation of Bach1. *Cell*. 2019;178(2):316-329.e18. doi: 10.1016/j.cell.2019.06.003
47. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO. Antioxidants accelerate lung cancer progression in mice. *Sci Transl Med*. 2014;6(221):221ra15. doi: 10.1126/scitranslmed.3007653
48. Klein EA, Thompson IM Jr, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 2011;306(14):1549-1556. doi: 10.1001/jama.2011.1437
49. Yasueda A, Urushima H, Ito T. Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review. *Integr Cancer Ther*. 2016;15(1):17-39. doi: 10.1177/1534735415610427
50. Watson J. Oxidants, antioxidants and the current incurability of metastatic cancers. *Open Biol*. 2013;3(1):120144. doi: 10.1098/rsob.120144
51. Kaźmierczak-Barańska J, Boguszewska K, Adamus--Grabicka A, Karwowski BT. Two Faces of Vitamin C--Antioxidative and Pro-Oxidative Agent. *Nutrients*. 2020;12(5):1501. doi: 10.3390/nu12051501
52. Chakraborthy A, Ramani P, Sherlin HJ, Premkumar P, Natesan A. Antioxidant and pro-oxidant activity of Vitamin C in oral environment. *Indian J Dent Res*. 2014;25(4):499-504. doi: 10.4103/0970-9290.142547
53. Corpe CP, Eck P, Wang J, Al-Hasani H, Levine M. Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. *J Biol Chem*. 2013;288(13):9092-9101. doi: 10.1074/jbc.M112.436790
54. Pena E, Roa FJ, Inostroza E, et al. Increased expression of mitochondrial sodium-coupled ascorbic acid transporter-2 (mitSVCT2) as a central feature in breast cancer. *Free Radic Biol. Med*. 2019;135:283-292. doi: 10.1016/j.freeradbiomed.2019.03.015
55. van der Reest J, Gottlieb E. Anti-cancer effects of vitamin C revisited. *Cell Res*. 2016;26(3):269-270. doi: 10.1038/cr.2016.7
56. Yun J, Mullarky E, Lu C, et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science*. 2015;350(6266):1391-1396. doi: 10.1126/science.aaa5004
57. Toohey JI. Dehydroascorbic acid as an anti-cancer agent. *Cancer Lett*. 2008;263(2):164-169. doi: 10.1016/j.canlet.2008.02.002
58. Lakhal-Littleton S, Robbins PA. The interplay between iron and oxygen homeostasis with a particular focus on the heart. *J Appl Physiol* (1985). 2017;123(4):967-973. doi: 10.1152/japplphysiol.00237.2017
59. Merrill JF, Thomson DM, Hardman SE, Hepworth SD, Willie S, Hancock CR. Iron deficiency causes a shift in AMP-activated protein kinase (AMPK) subunit composition in rat skeletal muscle. *Nutr Metab*. 2012;9(1):104. doi: 10.1186/1743-7075-9-104
60. Carter A, Racey S, Veuger S. The Role of Iron in DNA and Genomic Instability in Cancer, a Target for Iron Chelators That Can Induce ROS. *Applied Sciences*. 2022;12(19):10161. doi: 10.3390/app121910161
61. Kakhlon O, Cabantchik ZI. The labile iron pool: characterization, measurement, and participation in cellular processes(1). *Free Radic Biol Med*. 2002;33(8):1037-1046. doi: 10.1016/s0891-5849(02)01006-7.
62. Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology*. 2000;149(1):43-50. doi: 10.1016/s0300-483x(00)00231-6
63. Hou X, Shen W, Huang X, Ai Z, Zhang L. Ascorbic acid enhanced activation of oxygen by ferrous iron: A case of aerobic degradation of rhodamine B. *J Hazard Mater*. 2016;308:67-74. doi: 10.1016/j.jhazmat.2016.01.031

64. Xu G, Wang H, Li X, Huang R, Luo L. Recent progress on targeting ferroptosis for cancer therapy. *Biochem Pharmacol*. 2021;190:114584. doi: 10.1016/ j.bcp.2021.114584
65. Tang Z, Huang Z, Huang Y, et al. Ferroptosis: The Silver Lining of Cancer Therapy. *Front Cell Dev Biol*. 2021;9:765859. doi: 10.3389/fcell.2021.765859
66. Lane DJ, Richardson DR. The active role of vitamin C in mammalian iron metabolism: much more than just enhanced iron absorption! *Free Radic Biol Med*. 2014;75:69-83. doi: 10.1016/j.freeradbiomed.2014.07.007
67. Ahmed RMA, Husain NEOSA. Changes in Serum Iron, Total Iron Binding Capacity and Transferrin Saturation Percent in Sudanese Females Newly Diagnosed with Breast Cancer at Khartoum Oncology Hospital: A Case - Control Study. *Sudan Journal of Medical Sciences*. 2017;12(3):119-132. doi: 10.18502/sjms.v12i3.915.
68. Fadavi P, Nafisi N, Hariri R, et al. Serum Ferritin, Vitamin D and Pathological Factors in Breast Cancer Patients. *Med J Islam Repub Iran*. 2021;35:162. doi: 10.47176/mjiri.35.162
69. Xia J, Xu H, Zhang X, et al. Multiple Myeloma Tumor Cells are Selectively Killed by Pharmacologically-dosed Ascorbic Acid. *EBioMedicine*. 2017;18:41-49. doi: 10.1016/j.ebiom.2017.02.011
70. Zhong H, Agani F, Baccala AA, et al. Increased expression of hypoxia inducible factor-1alpha in rat and human prostate cancer. *Cancer Res*. 1998;58(23):5280-5284.
71. Stasinopoulos I, O'Brien DR, Bhujwalla ZM. Inflammation, but not hypoxia, mediated HIF-1alpha activation depends on COX-2. *Cancer Biol Ther*. 2009;8(1):31-35. doi: 10.4161/cbt.8.1.7079
72. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. 2003;3(10):721-732. doi: 10.1038/nrc1187
73. Soni S, Padwad YS. HIF-1 in cancer therapy: two decade long story of a transcription factor. *Acta Oncol*. 2017;56(4):503-515. doi: 10.1080/0284186X.2017.1301680
74. Jögi A, Ehinger A, Hartman L, Alkner S. Expression of HIF-1α is related to a poor prognosis and tamoxifen resistance in contralateral breast cancer. *PLoS ONE*. 2019;14(12):e0226150. doi: 10.1371/journal.pone.0226150
75. Jarman EJ, Ward C, Turnbull AK, et al. HER2 regulates HIF-2α and drives an increased hypoxic response in breast cancer. *Breast Cancer Res*. 2019;21(1):10. doi: 10.1186/s13058-019-1097-0
76. Burroughs SK, Kaluz S, Wang D, Wang K, Van Meir EG, Wang B. Hypoxia inducible factor pathway inhibitors as anticancer therapeutics. *Future Med Chem*. 2013;5(5):553-572. doi: 10.4155/fmc.13.17
77. Fallah J, Brave MH, Weinstock C, et al. FDA Approval Summary: Belzutifan for von Hippel-Lindau Disease-Associated Tumors. *Clin Cancer Res*. 2022;28(22):4843-4848. doi: 10.1158/1078-0432.CCR-22-1054
78. Miles SL, Fischer AP, Joshi SJ, Niles RM. Ascorbic acid and ascorbate-2-phosphate decrease HIF activity and malignant properties of human melanoma cells. *BMC Cancer*. 2015;15:867. doi: 10.1186/s12885-015-1878-5
79. Zhao L, Wang J, Zhang Y, Wang L, Yu M, Wang F. Vitamin C decreases VEGF expression levels via hypoxia-inducible factor-1α dependent and independent pathways in lens epithelial cells. *Mol Med Rep*. 2020;22(1):436-444. doi: 10.3892/mmr.2020.11103
80. Wohlrab C, Kuiper C, Vissers MC, Phillips E, Robinson BA, Dachs GU. Ascorbate modulates the hypoxic pathway by increasing intracellular activity of the HIF hydroxylases in renal cell carcinoma cells. *Hypoxia* (Auckl). 2019;7:17-31. doi: 10.2147/HP.S201643
81. Kuiper C, Dachs GU, Munn D, et al. Increased Tumor Ascorbate is Associated with Extended Disease-Free Survival and Decreased Hypoxia-Inducible Factor-1 Activation in Human Colorectal Cancer. *Front Oncol*. 2014;4:10. doi: 10.3389/fonc.2014.00010
82. Kuiper C, Molenaar IG, Dachs GU, Currie MJ, Sykes PH, Vissers MC. Low ascorbate levels are associated with increased hypoxia-inducible factor-1 activity and an aggressive tumor phenotype in endometrial cancer. *Cancer Res*. 2010;70(14):5749-5758. doi: 10.1158/0008-5472.CAN-10-0263
83. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30(7):1073-1081. doi: 10.1093/carcin/bgp127
84. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023. doi: 10.1038/sigtrans.2017.23
85. Wu Z, Li J, Zhang Y, Hu L, Peng X. Synchronous co-expression of Id-1 and nuclear NF-κB p65 promotes cervical cancer progression and malignancy, and is associated with a poor prognosis and chemosensitivity. *Oncol Rep*. 2019;42(5):2075-2086. doi: 10.3892/or.2019.7301
86. Liu AR, Ramakrishnan P. Regulation of Nuclear Factor-kappaB Function by O-GlcNAcylation in Inflammation and Cancer. *Front Cell Dev Biol*. 2021;9:751761. doi: 10.3389/fcell.2021.751761
87. Ramadass V, Vaiyapuri T, Tergaonkar V. Small Molecule NF-κB Pathway Inhibitors in Clinic. *Int J Mol Sci*. 2020;21(14):5164. doi: 10.3390/ijms21145164
88. Ameya Paranjpe, Kalkunte S. Srivenugopal, Degradation of NF-κB, p53 and other regulatory redox-sensitive proteins by thiol-conjugating and -nitrosylating drugs in human tumor cells, *Carcinogenesis*. 2013;(34)5:990-1000. doi: 10.1093/carcin/bgt032
89. Korashy HM, El-Kadi AO. The role of redox-sensitive transcription factors NF-kappaB and AP-1 in the modulation of the Cyp1a1 gene by mercury, lead, and copper. *Free Radic Biol Med*. 2008;44(5):795-806. doi: 10.1016/j.freeradbiomed.2007.11.003
90. Nakajima S, Kitamura M. Bidirectional regulation of NF-κB by reactive oxygen species: a role of unfolded prote-

in response. *Free Radic Biol Med*. 2013;65:162-174. doi: 10.1016/j.freeradbiomed.2013.06.020
91. Du YT, Long Y, Tang W, Liu XF, Dai F, Zhou B. Prooxidative inhibition against NF-κB-mediated inflammation by pharmacological vitamin C. *Free Radic Biol Med*. 2022;180:85-94. doi: 10.1016/j.freeradbiomed.2022.01.007
92. Cárcamo JM, Pedraza A, Bórquez-Ojeda O, Golde DW. Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation. *Biochemistry*. 2002;41(43):12995-3002. doi: 10.1021/bi0263210
93. Andrew G. Bowie, Luke A. J. O'Neill; Vitamin C Inhibits NF-κB Activation by TNF Via the Activation of p38 Mitogen-Activated Protein Kinase1. *J Immunol*. 2000;165(12):7180-7188.doi: 10.4049/jimmunol.165.12.7180
94. Cárcamo JM, Pedraza A, Bórquez-Ojeda O, Zhang B, Sanchez R, Golde DW. Vitamin C is a kinase inhibitor: dehydroascorbic acid inhibits IkappaBalpha kinase beta. *Mol Cell Biol*. 2004;24(15):6645-6652. doi: 10.1128/MCB.24.15.6645-6652.2004
95. Morante-Palacios O, Godoy-Tena G, Calafell-Segura J, et al. Vitamin C enhances NF-κB-driven epigenomic reprogramming and boosts the immunogenic properties of dendritic cells. *Nucleic Acids Res*. 2022;50(19):10981-10994. doi: 10.1093/nar/gkac941
96. Morante-Palacios O, Godoy-Tena G, Calafell-Segura J, et al. Vitamin C triggers NF-κB-driven epigenomic reprogramming and enhanced immunogenic responses of dendritic cells. *bioRxiv* 2022;05(26):493381; doi: 10.1101/2022.05.26.493381
97. Tas SW, de Jong EC, Hajji N, et al. Selective inhibition of NF-kappaB in dendritic cells by the NEMO-binding domain peptide blocks maturation and prevents T cell proliferation and polarization. *Eur J Immunol*. 2005;35(4):1164-1174. doi: 10.1002/eji.200425956
98. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov*. 2017;7(8):818-831. doi: 10.1158/2159-8290.CD-17-0151
99. Ferrone CK, Blydt-Hansen M, Rauh MJ. Age-Associated TET2 Mutations: Common Drivers of Myeloid Dysfunction, Cancer and Cardiovascular Disease. *Int J Mol Sci*. 2020;21(2):626. doi: 10.3390/ijms21020626
100. Huang F, Sun J, Chen W, et al. TET2 deficiency promotes MDS-associated leukemogenesis. *Blood Cancer J*. 2022;12(10):141. doi: 10.1038/s41408-022-00739-w
101. Wang R, Gao X, Yu L. The prognostic impact of tet oncogene family member 2 mutations in patients with acute myeloid leukemia: a systematic-review and meta-analysis. *BMC Cancer*. 2019;19(1):389. doi: 10.1186/s12885-019-5602-8
102. Mingay M, Chaturvedi A, Bilenky M, et al. Vitamin C-induced epigenomic remodelling in IDH1 mutant acute myeloid leukaemia. *Leukemia*. 2018;32(1):11-20. doi: 10.1038/leu.2017.171
103. Derissen EJ, Beijnen JH, Schellens JH. Concise drug review: azacitidine and decitabine. *Oncologist*. 2013;18(5):619-24. doi: 10.1634/theoncologist.2012-0465
104. Liu M, Ohtani H, Zhou W, et al. Vitamin C increases viral mimicry induced by 5-aza-2'-deoxycytidine. *Proc Natl Acad Sci USA*. 2016;113(37):10238-11244. doi: 10.1073/pnas.1612262113
105. Bejar R, Lord A, Stevenson K, Bar-Natan M, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood*. 2014;124(17):2705-2712. doi: 10.1182/blood-2014-06-582809
106. Gerecke C, Schumacher F, Edlich A, et al. Vitamin C promotes decitabine or azacytidine induced DNA hydroxymethylation and subsequent reactivation of the epigenetically silenced tumour suppressor CDKN1A in colon cancer cells. *Oncotarget*. 2018;9(67):32822-32840. doi: 10.18632/oncotarget.25999
107. Gillberg L, Ørskov AD, Nasif A, et al. Oral vitamin C supplementation to patients with myeloid cancer on azacitidine treatment: Normalization of plasma vitamin C induces epigenetic changes. *Clin Epigenetics*. 2019;11(1):143. doi: 10.1186/s13148-019-0739-5
108. Joshi K, Zhang L, Breslin S J P, Kini AR, Zhang J. Role of TET dioxygenases in the regulation of both normal and pathological hematopoiesis. *J Exp Clin Cancer Res*. 2022;41(1):294. doi: 10.1186/s13046-022-02496-x
109. Agathocleous M, Meacham CE, Burgess RJ, et al. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. *Nature*. 2017;549(7673):476-481. doi: 10.1038/nature23876
110. Cimmino L, Dolgalev I, Wang Y, et al. Restoration of TET2 Function Blocks Aberrant Self-Renewal and Leukemia Progression. *Cell*. 2017;170(6):1079-1095.e20. doi: 10.1016/j.cell.2017.07.032
111. Miller PG, Ebert BL. Leukaemia: Vitamin C regulates stem cells and cancer. *Nature*. 2017;549(7673):462-464. doi: 10.1038/nature23548
112. Phadke I, Pouzolles M, Machado A, et al. Vitamin C deficiency reveals developmental differences between neonatal and adult hematopoiesis. *Front Immunol*. 2022;13:898827. doi: 10.3389/fimmu.2022.898827
113. Taira A, Palin K, Kuosmanen A, et al. Vitamin C boosts DNA demethylation in TET2 germline mutation carriers. *Clin Epigenetics*. 2023;15(1):7. doi: 10.1186/s13148-022-01404-6
114. Upadhyay S, Sharma N, Gupta KB, Dhiman M. Role of immune system in tumor progression and carcinogenesis. *J Cell Biochem*. 2018;119(7):5028-5042. doi: 10.1002/jcb.26663
115. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev*. 2018;32(19-20):1267-1284. doi: 10.1101/gad.314617.118
116. Cai X, Zhan H, Ye Y, et al. Current Progress and Future Perspectives of Immune Checkpoint in Cancer and

Infectious Diseases. *Front Genet*. 2021;12:785153. doi: 10.3389/fgene.2021.785153

117. Mehdizadeh S, Bayatipoor H, Pashangzadeh S, Jafarpour R, Shojaei Z, Motallebnezhad M. Immune checkpoints and cancer development: Therapeutic implications and future directions. *Pathol Res Pract*. 2021;223:153485. doi: 10.1016/j.prp.2021.153485
118. Lipson EJ, Drake CG. Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. *Clin Cancer Res*. 2011;17(22):6958-6962. doi: 10.1158/1078-0432.CCR-11-1595
119. Klemen ND, Wang M, Feingold PL, et al. Patterns of failure after immunotherapy with checkpoint inhibitors predict durable progression-free survival after local therapy for metastatic melanoma. *J Immunother Cancer*. 2019;7(1):196. doi: 10.1186/s40425-019-0672-3
120. Johns AC, Wei L, Grogan M, et al. Checkpoint inhibitor immunotherapy toxicity and overall survival among older adults with advanced cancer. *J Geriatr Oncol*. 2021;12(5):813-819. doi: 10.1016/j.jgo.2021.02.002
121. Schoenfeld JD, Alexander MS, Waldron TJ, et al. Pharmacological Ascorbate as a Means of Sensitizing Cancer Cells to Radio-Chemotherapy While Protecting Normal Tissue. *Semin Radiat Oncol*. 2019;29(1):25-32. doi: 10.1016/j.semradonc.2018.10.006
122. Burkard M, Niessner H, Leischner C, et al. High-Dose Ascorbate in Combination with Anti-PD1 Checkpoint Inhibition as Treatment Option for Malignant Melanoma. *Cells*. 2023;12(2):254. doi: 10.3390/cells12020254
123. Luchtel RA, Bhagat T, Pradhan K, et al. High-dose ascorbic acid synergizes with anti-PD1 in a lymphoma mouse model. *Proc Natl Acad Sci USA*. 2020;117(3):1666-1677. doi: 10.1073/pnas.1908158117
124. Magrì A, Germano G, Lorenzato A, et al. High-dose vitamin C enhances cancer immunotherapy. *Sci Transl Med*. 2020;12(532):eaay8707. doi: 10.1126/scitranslmed.aay8707
125. Peng D, He A, He S, et al. Ascorbic acid induced TET2 enzyme activation enhances cancer immunotherapy efficacy in renal cell carcinoma. *Int J Biol Sci*. 2022;18(3):995-1007. doi: 10.7150/ijbs.67329
126. Oberritter H, Glatthaar B, Moser U, Schmidt KH. Effect of functional stimulation on ascorbate content in phagocytes under physiological and pathological conditions. *Int Arch Allergy Appl Immunol*. 1986;81(1):46-50. doi: 10.1159/000234106
127. Bozonet SM, Carr AC, Pullar JM, Vissers MC. Enhanced human neutrophil vitamin C status, chemotaxis and oxidant generation following dietary supplementation with vitamin C-rich SunGold kiwifruit. *Nutrients*. 2015;7(4):2574-2588. doi: 10.3390/nu7042574
128. Washko PW, Wang Y, Levine M. Ascorbic acid recycling in human neutrophils. *J Biol Chem*. 1993;25,268(21):15531-15535.
129. Masucci MT, Minopoli M, Del Vecchio S, Carriero MV. The Emerging Role of Neutrophil Extracellular Traps (NETs) in Tumor Progression and Metastasis. *Front Immunol*. 2020;11:1749. doi: 10.3389/fimmu.2020.01749
130. Kaltenmeier C, Yazdani HO, Morder K, Geller DA, Simmons RL, Tohme S. Neutrophil Extracellular Traps Promote T Cell Exhaustion in the Tumor Microenvironment. *Front Immunol*. 2021;12:785222. doi: 10.3389/fimmu.2021.785222
131. Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007;176(2):231-241. doi: 10.1083/jcb.200606027
132. Mohammed BM, Fisher BJ, Kraskauskas D, et al. Vitamin C: a novel regulator of neutrophil extracellular trap formation. *Nutrients*. 2013;5(8):3131-3151.
133. Sae-Khow K, Tachaboon S, Wright HL, et al. Defective Neutrophil Function in Patients with Sepsis Is Mostly Restored by ex vivo Ascorbate Incubation. *J Inflamm Res*. 2020;13:263-274. doi: 10.2147/JIR.S252433
134. Van Gorkom GNY, Klein Wolterink RGJ, Van Elssen CHMJ, Wieten L, Germeraad WTV, Bos GMJ. Influence of Vitamin C on Lymphocytes: An Overview. *Antioxidants*. 2018;7(3):41. doi: 10.3390/antiox7030041
135. Bergsten P, Yu R, Kehrl J, Levine M. Ascorbic acid transport and distribution in human B lymphocytes. *Arch Biochem Biophys*. 1995;317(1):208-214. doi: 10.1006/abbi.1995.1155
136. Manning J, Mitchell B, Appadurai DA et al. Vitamin C promotes maturation of T-cells. *Antioxid Redox Signal*. 2013;19(17):2054-2067. doi: 10.1089/ars.2012.4988
137. Wu CY, Zhang B, Kim H, Anderson SK, Miller JS, Cichocki F. Ascorbic Acid Promotes KIR Demethylation during Early NK Cell Differentiation. *J Immunol*. 2020;205(6):1513-1523. doi: 10.4049/jimmunol.2000212
138. Ichiyama K, Mitsuzumi H, Zhong M, et al. Promotion of IL-4- and IL-5-dependent differentiation of anti-mu-primed B cells by ascorbic acid 2-glucoside. *Immunol Lett*. 2009;122(2):219-226. doi: 10.1016/j.imlet.2009.01.007
139. Heuser G, Vojdani A. Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase-C. *Immunopharmacol Immunotoxicol*. 1997;19(3):291-312.
140. Kouakanou L, Wang H, Sun G, et al. Vitamin C treatment prevents CAR T cell exhaustion, maintains stem cell phenotype and enhances antitumor function. *Journal for ImmunoTherapy of Cancer*. 2022;10(2):A370. doi: 10.1136/jitc-2022-SITC2022.0351.
141. Kouakanou L, Peters C, Brown CE, Kabelitz D, Wang LD. Vitamin C, From Supplement to Treatment: A Re-Emerging Adjunct for Cancer Immunotherapy? *Front Immunol*. 2021;12:765906. doi: 10.3389/fimmu.2021.765906
142. Huijskens MJ, Walczak M, Sarkar S, et al. Ascorbic acid promotes proliferation of natural killer cell populations in culture systems applicable for natural killer cell therapy. *Cytotherapy*. 2015;17(5):613-620. doi: 10.1016/j.jcyt.2015.01.004

143. Saura-Esteller J, de Jong M, King LA, et al. Gamma Delta T-Cell Based Cancer Immunotherapy: Past-Present-Future. *Front Immunol*. 2022;13:915837. doi: 10.3389/fimmu.2022.915837
144. Healio. https://www.healio.com/news/hematology-oncology/20220422/fda-grants-fast-track-designation-to-gammadelta-tcell-therapy-for-advanced-lymphoma. Accessed January 23, 2023.
145. Nishimoto KP, Barca T, Azameera A, et al. Allogeneic CD20-targeted γδ T cells exhibit innate and adaptive antitumor activities in preclinical B-cell lymphoma models. *Clin Transl Immunology*. 2022;11(2):e1373. doi: 10.1002/cti2.1373
146. Neelapu SS, Stevens DA, Hamadani M, et al. A Phase 1 Study of ADI-001: Anti-CD20 CAR-Engineered Allogeneic Gamma Delta1 (γδ) T Cells in Adults with B--Cell Malignancies. *Blood*. 2022;140(1):4617-4619. doi: 10.1182/blood-2022-157400.
147. Kouakanou L, Xu Y, Peters C, et al. Vitamin C promotes the proliferation and effector functions of human γδ T cells. *Cell Mol Immunol.* 2020;17(5):462-473. doi: 10.1038/s41423-019-0247-8
148. Xu Y, Xiang Z, Alnaggar M, et al. Allogeneic Vγ9Vδ2 T--cell immunotherapy exhibits promising clinical safety and prolongs the survival of patients with late-stage lung or liver cancer. *Cell Mol Immunol.* 2021;18(2):427-439. doi: 10.1038/s41423-020-0515-7
149. Padayatty SJ, Sun AY, Chen Q, Espey MG, Drisko J, Levine M. Vitamin C: intravenous use by complementary and alternative medicine practitioners and adverse effects. *PLoS One*. 2010;5(7):e11414. doi: 10.1371/journal.pone.0011414
150. Zasowska-Nowak A, Nowak PJ, Ciałkowska-Rysz A. High-Dose Vitamin C in Advanced-Stage Cancer Patients. *Nutrients*. 2021;13(3):735. doi: 10.3390/nu13030735
151. Mohseni S, Tabatabaei-Malazy O, Ejtahed HS, et al. Effect of vitamins C and E on cancer survival; a systematic review. *Daru.* 2022;30(2):427-441. doi: 10.1007/s40199-022-00451-x
152. Nielsen TK, Højgaard M, Andersen JT, et al. Weekly ascorbic acid infusion in castration-resistant prostate cancer patients: a single-arm phase II trial. *Transl Androl Urol*. 2017;6(3):517-528. doi: 10.21037/tau.2017.04.42
153. Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. *Cancer Chemother Pharmacol*. 2013;72(1):139-146. doi: 10.1007/s00280-013-2179-9
154. Hoffer LJ, Levine M, Assouline S, et al . Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. *Ann Oncol.* 2008;19(11):1969-1974. doi: 10.1093/annonc/mdn377
155. Padayatty SJ, Riordan HD Hewitt SM, et al. Intravenously administered vitamin C as cancer therapy: three cases. *CMAJ.* 2006;174(7):937-942. doi: 10.1503/cmaj.050346
156. Seo MS, Kim JK, Shim JY. High-Dose Vitamin C Promotes Regression of Multiple Pulmonary Metastases Originating from Hepatocellular Carcinoma. *Yonsei Med J*. 2015;56(5):1449-1452. doi: 10.3349/ymj.2015.56.5.1449
157. Drisko JA, Serrano OK, Spruce LR, Chen Q, Levine M. Treatment of pancreatic cancer with intravenous vitamin C: a case report. *Anticancer Drugs*. 2018;29(4):373-379. doi: 10.1097/CAD.0000000000000603
158. Raymond YC, Glenda CS, Meng LK. Effects of High Doses of Vitamin C on Cancer Patients in Singapore: Nine Cases. *Integr Cancer Ther.* 2016;15(2):197-204. doi: 10.1177/1534735415622010
159. Das AB, Kakadia PM, Wojcik D, et al. Clinical remission following ascorbate treatment in a case of acute myeloid leukemia with mutations in TET2 and WT1. *Blood Cancer J*. 2019;9(10):82. doi: 10.1038/s41408-019-0242-4
160. ClinicalTrials.gov Identifier: NCT03682029. Epigenetics, Vitamin C, and Abnormal Blood Cell Formation - Vitamin C in Patients with Low-Risk Myeloid Malignancies (EVITA). Accessed February 2, 2023.
161. ClinicalTrials.gov Identifier: NCT03613727. Therapeutic Use of Intravenous Vitamin C in Allogeneic Stem Cell Transplant Recipients. Accessed February 2, 2023.
162. Wang F, He MM, Wang ZX, et al. Phase I study of high-dose ascorbic acid with mFOLFOX6 or FOLFIRI in patients with metastatic colorectal cancer or gastric cancer. *BMC Cancer*. 2019;19(1):460. doi: 10.1186/s12885-019-5696-z
163. Wang F, He MM, Xiao J, et al. A Randomized, Open--Label, Multicenter, Phase 3 Study of High-Dose Vitamin C Plus FOLFOX ± Bevacizumab versus FOLFOX ± Bevacizumab in Unresectable Untreated Metastatic Colorectal Cancer (VITALITY Study). *Clin Cancer Res.* 2022;28(19):4232-4239. doi: 10.1158/1078-0432.CCR-22-0655
164. Welsh JL, Wagner BA, van't Erve TJ, et al. Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial. *Cancer Chemother Pharmacol*. 2013;71(3):765-775. doi: 10.1007/s00280-013-2070-8
165. ClinicalTrials.gov Identifier: NCT02905578. A Phase 2 Trial of High-dose Ascorbate for Pancreatic Cancer (PACMAN 2.1). Accessed January 23, 2023.
166. Polireddy K, Dong R, Reed G, et al. High Dose Parenteral Ascorbate Inhibited Pancreatic Cancer Growth and Metastasis: Mechanisms and a Phase I/IIa study. *Sci Rep.* 2017;7(1):17188. doi: 10.1038/s41598-017-17568-8
167. Yun J, Mullarky E, Lu C, et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science*. 2015;350(6266):1391-1396. doi: 10.1126/science.aaa5004
168. Wang S, Zheng Y, Yang F, et al. The molecular biology of pancreatic adenocarcinoma: translational challenges and clinical perspectives. *Signal Transduct Target Ther*. 2021;6(1):249. doi: 10.1038/s41392-021-00659-4

169. Uetaki M, Tabata S, Nakasuka F, Soga T, Tomita M. Metabolomic alterations in human cancer cells by vitamin C-induced oxidative stress. *Sci Rep*. 2015;5:13896. doi: 10.1038/srep13896
170. Kim JH, Hwang S, Lee JH, Im SS, Son J. Vitamin C Suppresses Pancreatic Carcinogenesis through the Inhibition of Both Glucose Metabolism and Wnt Signaling. *Int J Mol Sci*. 2022;23(20):12249. doi: 10.3390/ijms232012249
171. Banella C, Catalano G, Travaglini S, et al. Ascorbate Plus Buformin in AML: A Metabolic Targeted Treatment. *Cancers* (Basel). 2022;14(10):2565. doi: 10.3390/cancers14102565
172. ClinicalTrials.gov Identifier: NCT04033107. High Dose Vitamin C Combined With Metformin in the Treatment of Malignant Tumors. Accessed January 23, 2023.
173. Qian W, Wang L, Li P, et al. Efficiency and Tolerability of Induction and Consolidation Therapy with Arsenic Trioxide/Bortezomib/Ascorbic Acid/Dexamethasone (ABCD) Regimen Compared to Bortezomib/Dexamethasone (BD) Regimen in Newly Diagnosed Myeloma Patients. *Cancer Manag Res*. 2020;12:431-441. doi: 10.2147/CMAR.S212455
174. Berenson JR, Matous J, Swift RA, Mapes R, Morrison B, Yeh HS. A phase I/II study of arsenic trioxide/bortezomib/ascorbic acid combination therapy for the treatment of relapsed or refractory multiple myeloma. *Clin Cancer Res*. 2007;13(6):1762-1768. doi: 10.1158/1078-0432.CCR-06-1812
175. Berenson JR, Boccia R, Siegel D, et al. Efficacy and safety of melphalan, arsenic trioxide and ascorbic acid combination therapy in patients with relapsed or refractory multiple myeloma: a prospective, multicentre, phase II, single-arm study. *Br J Haematol*. 2006;135(2):174-83. doi: 10.1111/j.1365-2141.2006.06280.x
176. Gill H, Kumana CR, Yim R, et al. Oral arsenic trioxide incorporation into frontline treatment with all-trans retinoic acid and chemotherapy in newly diagnosed acute promyelocytic leukemia: A 5-year prospective study. *Cancer*. 2019;125(17):3001-3012. doi: 10.1002/cncr.32180
177. Gill HS, Yim R, Kumana CR, Tse E, Kwong YL. Oral arsenic trioxide, all-trans retinoic acid, and ascorbic acid maintenance after first complete remission in acute promyelocytic leukemia: Long-term results and unique prognostic indicators. *Cancer*. 2020; 126(14):3244-3254. doi: 10.1002/cncr.32937
178. Gill H, Raghupathy R, Ni M, et al. Epidemiology and outcomes of acute promyelocytic leukaemia in the era of all-trans retinoic acid (ATRA) chemotherapy and arsenic trioxide plus ATRA: a retrospective analysis. *Lancet Oncology*. 2022;23(1):11. doi: 10.1016/S1470-2045(22)00410-7
179. ClinicalTrials.gov Identifier: NCT04251754. The Acute Promyelocytic Leukaemia Asian Consortium (APL-AC) Project. Accessed January 23, 2023.
180. ClinicalTrials.gov Identifier: NCT03624270. Oral Arsenic Trioxide for Newly Diagnosed Acute Promyelocytic Leukaemia. Accessed January 23, 2023.
181. Zhao H, Zhu H, Huang J et al. The synergy of Vitamin C with decitabine activates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid leukemia. *Leuk Res*. 2018;66:1-7. doi: 10.1016/j.leukres.2017.12.009
182. Welch JS, Klco JM, Gao F, et al. Combination decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia: a phase I study. *Am J Hematol*. 2011;86(9):796-800. doi: 10.1002/ajh.22092
183. Zhang X, Fu R, Yu J, Wu X. DNA demethylation: where genetics meets epigenetics. *Curr Pharm Des*. 2014;20(11):1625-1631. doi: 10.2174/13816128113199990546
184. ACTRN12621000223831: Precision Medicine for Chronic Myelomonocytic Leukaemia in Adults: A phase II Trial Studying the Efficacy of Lenzilumab and High Dose Ascorbate with Azacitidine Based on Molecular Profiling. https://anzctr.org.au/Trial/Registration/TrialReview.aspx?id=380941&isReview=true. Accessed February 10, 2023.
185. ClinicalTrials.gov Identifier: NCT03999723. Combining Active and Passive DNA Hypomethylation (EVI-3). Accessed January 23, 2023.
186. Böttger F, Vallés-Martí A, Cahn L, Jimenez CR. High-dose intravenous vitamin C, a promising multi-targeting agent in the treatment of cancer. *J Exp Clin Cancer Res*. 2021;40(1):343. doi: 10.1186/s13046-021-02134-y
187. Bedhiafi T, Inchakalody VP, Fernandes Q, et al. The potential role of vitamin C in empowering cancer immunotherapy. *Biomed Pharmacother*. 2022;146:112553. doi: 10.1016/j.biopha.2021.112553
188. Darwiche W, Gomila C, Ouled-Haddou H, et al. Ascorbic acid (vitamin C) synergistically enhances the therapeutic effect of targeted therapy in chronic lymphocytic leukemia. *J Exp Clin Cancer Res*. 2020;39(1):228. doi: 10.1186/s13046-020-01738-0
189. Dhahri A and Chhabra G. The Abating Effect of Vitamin-C on Imatinib in a CML Patient: A First Case Report. *Austin J Med Oncol*. 2020;7(3):1056.
190. Heaney ML, Gardner JR, Karasavvas N, et al. Vitamin C antagonizes the cytotoxic effects of antineoplastic drugs. *Cancer Res*. 2008;68(19):8031-8038. doi: 10.1158/0008-5472.CAN-08-1490
191. Perrone G, Hideshima T, Ikeda H, et al. Ascorbic acid inhibits antitumor activity of bortezomib in vivo. *Leukemia*. 2009;23:1679-1686.
192. Jia L, Liu FT. Why bortezomib cannot go with 'green'? *Cancer Biol Med*. 2013;10(4):206-213. doi: 10.7497/j.issn.2095-3941.2013.04.004
193. Bolaman AZ, Turgutkaya A, Küçükdiler HE, Selim C, Yavaşoğlu İ. Pharmacological dose ascorbic acid administration in relapsed refractory multiple myeloma patients. *Leuk Res Rep*. 2021;16:100281. doi: 10.1016/j.lrr.2021.100281

194. White R, Nonis M, Pearson JF, et al. Low Vitamin C Status in Patients with Cancer Is Associated with Patient and Tumor Characteristics. *Nutrients*. 2020;12(8):2338. doi: 10.3390/nu12082338
195. Klimant E, Wright H, Rubin D, Seely D, Markman M. Intravenous vitamin C in the supportive care of cancer patients: a review and rational approach. *Curr Oncol*. 2018;25(2):139-148. doi: 10.3747/co.25.3790
196. Hoffer LJ, Robitaille L, Zakarian R, et al. High-dose intravenous vitamin C combined with cytotoxic chemotherapy in patients with advanced cancer: a phase I-II clinical trial. *PLoS One*. 2015;10(4):e0120228. doi: 10.1371/journal.pone.0120228
197. Cabanillas F. Vitamin C and cancer: what can we conclude-1,609 patients and 33 years later? *P R Health Sci J*. 2010;29(3):215-217.
198. Saul AW. Vitamin C and cancer: what can we conclude--1,609 patients and 33 years later: comment on the article by Cabanillas. *P R Health Sci J*. 2010;29(4):409-410.
199. González MJ, Miranda-Massari JR, Duconge J. Vitamin C and cancer: what can we conclude--1,609 patients and 33 years later: comment on the article by Cabanillas. *P R Health Sci J*. 2010;29(4):410-411.