OPINION

Targeting cancer vulnerabilities with high-dose vitamin C

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Abstract | Over the past century, the notion that vitamin C can be used to treat cancer has generated much controversy. However, new knowledge regarding the pharmacokinetic properties of vitamin C and recent high-profile preclinical studies have revived interest in the utilization of high-dose vitamin C for cancer treatment. Studies have shown that pharmacological vitamin C targets many of the mechanisms that cancer cells utilize for their survival and growth. In this Opinion article, we discuss how vitamin C can target three vulnerabilities many cancer cells share: redox imbalance, epigenetic reprogramming and oxygen-sensing regulation. Although the mechanisms and predictive biomarkers that we discuss need to be validated in well-controlled clinical trials, these new discoveries regarding the anticancer properties of vitamin C are promising to help identify patient populations that may benefit the most from high-dose vitamin C therapy, developing effective combination strategies and improving the overall design of future vitamin C clinical trials for various types of cancer.

The 'magic bullet' theory serves as a paradigm for modern cancer research and has inspired numerous ground-breaking targeted therapies such as imatinib and vemurafenib[1]. However, despite remarkable initial responses, the eventual acquisition of resistance and therapy-associated toxic effects continues to impede progress towards achieving meaningful patient survival. Thus, a new strategy for treating and managing cancer is needed. In this Opinion article, we propose that vitamin C, a natural compound with an unusually high safety profile, can be used to target multiple critical pathways in cancer.

The utilization of high-dose vitamin C as a cancer therapy has a controversial history. Much of this controversy stems from conflicting results in early clinical trials, as well as the lack of biomarkers and a clear understanding of vitamin C's mechanism of action. Despite this, publications over the past 40 years suggest that these contradictory results can be explained, at least in part, by differences in the administration route of vitamin C; the millimolar concentration of vitamin C cytotoxic to cancer cells is achievable only by intravenous injection, not by oral administration[2]. As a result, there are approximately a dozen ongoing clinical trials exploring the safety and efficacy of intravenous high-dose vitamin C for treating various types of cancer as a monotherapy or combination therapy[3] (TABLE 1). Given the revived clinical interest in vitamin C as a cancer therapy, this Opinion article examines the evidence supporting the therapeutic potential of vitamin C and highlights advances in the current understanding of its mechanisms of action. First, we summarize the biological functions and chemical properties of vitamin C. Second, we examine three different mechanisms by which high-dose vitamin C can selectively kill cancer cells. Understanding the multiple targets and mechanisms by which vitamin C exerts anticancer effects will be essential for identifying predictive biomarkers for patient stratification and developing potent combination strategies that lead to durable remission. Finally, we close our Review by sharing our perspective on the future of vitamin C research as a treatment for cancer.

Biology of vitamin C

Synthesis of vitamin C

Vitamin C is a six-carbon ketolactone synthesized from glucose by most animals in the kidney or liver[4]. However, humans — as well as other primates, guinea pigs and fruit bats — are unable to synthesize vitamin C because they harbour inactivating mutations in the gene encoding L-gulonolactone oxidase (GULO), the enzyme responsible for catalysing the last step of vitamin C synthesis[5]. Owing to this 'inborn metabolic error', humans must acquire vitamin C from dietary sources. The current recommended daily allowance of vitamin C (75–90 mg per day) can easily be achieved by consuming a balanced diet consisting of fruits and vegetables, yielding a plasma ascorbate concentration of 30–80 μM (REF.[4]). By contrast, sustained malnutrition or low dietary vitamin C intake will lead to plasma levels below 10 μM and result in scurvy, a vitamin C deficiency disease characterized by bleeding gums, impaired wound healing, anaemia, fatigue, depression and, in severe cases, death[4].

Redox forms of vitamin C

Vitamin C exists in different redox forms depending on the biological conditions (FIG. 1a). Fully reduced vitamin C (ascorbate or ascorbic acid) can be oxidized both intracellularly and extracellularly. Extracellular ascorbate is oxidized by free radicals or reactive oxygen species (ROS) producing a weak radical intermediate, ascorbate radical ($Asc^{\bullet-}$), which is then oxidized fully into dehydroascorbic acid (DHA)[6]. DHA, having a short half-life (less than 1 minute)[7], accounts for only approximately 1–5% of vitamin C in the human body[4] and is either transported inside the cell (BOX 1) or becomes irreversibly hydrolysed into 2,3-L-diketoglutonate (2,3-DKG). 2,3-DKG is then degraded into oxalic acid and threonic acid, resulting in a net loss of vitamin C[8]. Inside the cell, DHA is rapidly reduced back to ascorbate by reacting with a reduced glutathione (GSH)[8]. Oxidized glutathione (glutathione disulfide (GSSG)) is then recycled back to GSH by NADPH[8].

Table 1 | **List of active intravenous vitamin C clinical trials as an anticancer therapy**

Phase	Trial name	Institution	Trial design	Cancer type	Drugs	Refs
Phase I	Gemcitabine, ascorbate, radiation therapy for pancreatic cancer	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Single arm	Pancreatic neoplasms	Gemcitabine; radiation + IV ascorbate	142
Phase I	High-dose ascorbate in glioblastoma multiforme	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Single arm	Glioblastoma	Radiation + temozolomide + IV ascorbate	143
Phase I/II	High-dose ascorbate + nanoparticle paclitaxel protein bound + cisplatin + gemcitabine in patients who have had no prior therapy for their metastatic pancreatic cancer	Piedmont Cancer Institute (USA)	Single arm	Metastatic pancreatic cancer	Paclitaxel, cisplatin, gemcitabine + IV ascorbate	144
Phase I/II	High-dose ascorbate + nanoparticle paclitaxel protein bound + cisplatin + gemcitabine in patients who have no prior therapy for their metastatic pancreatic cancer	HonorHealth Research Institute, University of California–San Diego Moores Cancer Center and Piedmont Cancer Institute (USA)	Single arm	Pancreatic cancer	Paclitaxel protein-bound, cisplatin + IV ascorbate	145
Phase II	High-dose ascorbate in stage IV non-small-cell lung cancer	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Single arm	Non-small-cell lung cancer	Paclitaxel; carboplatin + IV ascorbate	146
Phase II	Therapeutic use of IV vitamin C in allogeneic stem cell transplant recipients	Virginia Commonwealth University–Massey Cancer Center (USA)	Single arm	Hodgkin lymphoma, lymphoid leukaemia and multiple myeloma	IV and oral vitamin C	147
Phase II	Pharmacological ascorbate combined with radiation and temozolomide in glioblastoma multiforme: a phase II trial	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Single arm	Glioblastoma multiforme	Radiation + temozolomide + IV ascorbate	148
Phase II	High-dose vitamin C IV infusion in patients with resectable or metastatic solid tumor malignancies	Weill Cornell Medicine (USA)	Single arm	Colorectal, pancreatic and lung cancer with *KRAS* or *BRAF* mutation	IV ascorbate	149
Phase II	Pharmacological ascorbate with concurrent chemotherapy and radiation therapy for non-small-cell lung cancer	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Single arm	Non-small-cell lung cancer	Radiation, paclitaxel, carboplatin + IV ascorbate	150
Phase II	Pharmacological ascorbate, gemcitabine, nab-paclitaxel for metastatic pancreatic cancer	Holden Comprehensive Cancer Center at the University of Iowa (USA)	Randomized two-arm	Pancreatic neoplasms	Paclitaxel, gemcitabine + IV ascorbate	151
Phase II	Ascorbic acid in combination with docetaxel in men with metastatic prostate cancer	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (USA)	Randomized two-arm	Hormone-resistant prostate cancer, metastatic prostate carcinoma and stage IV prostate cancer	IV ascorbate + docetaxel	152
Phase II	IV ascorbic acid as an adjunct to pazopanib in the first-line setting for metastatic or unresectable clear cell renal cell carcinoma (ccRCC)	Mayo Clinic in Florida and Minnesota, Illinois CancerCare-Peoria, Iowa-Wide Oncology Research Coalition NCORP and Sanford Medical Center Fargo (USA)	Randomized two-arm	Clear cell renal cell carcinoma	Pazopanib hydrochloride + IV ascorbate	153
Phase II	Ascorbic acid and combination chemotherapy in treating patients with relapsed or refractory lymphoma	Mayo Clinic in Arizona, Minnesota and Florida and Holden Comprehensive Cancer Center at the University of Iowa (USA)	Randomized two-arm	B cell lymphoma with MYC and BCL2/BCL6 rearrangement, and recurrent Hodgkin lymphoma	Carboplatin, cisplatin + IV ascorbate	154
Phase III	IV ascorbic acid in advanced gastric cancer	Sun Yat-Sen University Cancer Center (China)	Randomized two-arm	Gastric cancer	mFOLFOX6 + IV ascorbate	155
Phase III	IV ascorbic acid in combination with FOLFOX$^{+/-}$ bevacizumab versus treatment with FOLFOX$^{+/-}$ bevacizumab alone as first-line therapy for advanced colorectal cancer	Sun Yat-Sen University Cancer Center (China)	Randomized two-arm	Colorectal neoplasms	mFOLFOX6, bevacizuman + IV ascorbate	156

IV, intravenous.

Biological functions of vitamin C

The biological functions of vitamin C can be attributed to its biochemical property as an electron donor. Acting as an antioxidant, physiological ascorbate at micromolar concentrations can reduce harmful ROS[9]. Paradoxically, it can also function as a pro-oxidant at millimolar plasma concentrations, which can be achieved by intravenous administration of pharmacological ascorbate[4]. In addition to its redox functions, vitamin C affects iron metabolism by increasing ferritin

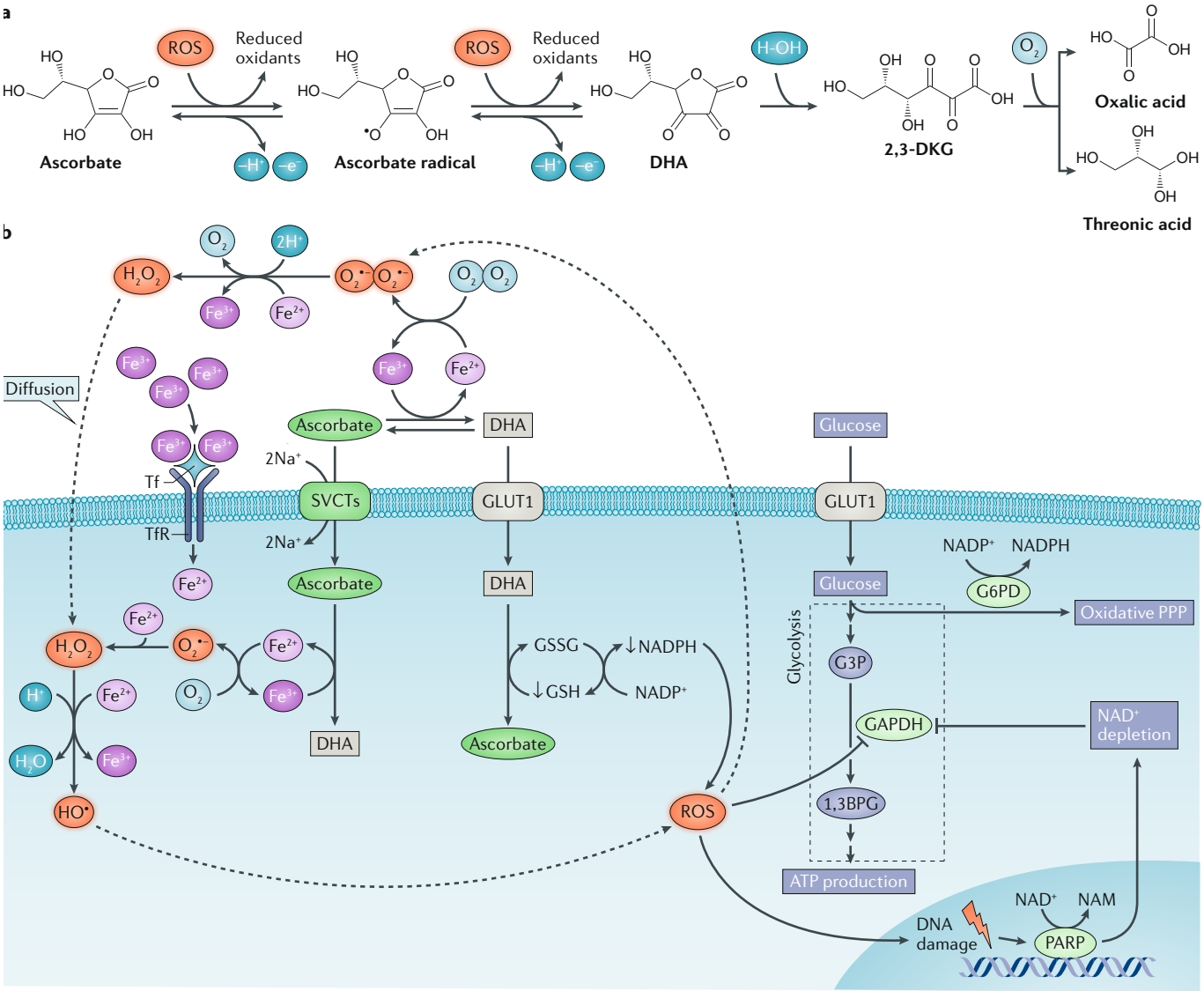

Fig. 1 | **Integrated pro-oxidant mechanism of vitamin C and cancer cell cytotoxicity. a** | Ascorbate can be oxidized in the extracellular space by reactive oxygen species (ROS), producing ascorbate radical, which can be oxidized to dehydroascorbic acid (DHA). DHA can be taken up by cells or irreversibly converted to 2,3-L-diketoglutonate (2,3-DKG), which is degraded into oxalic acid and threonic acid. **b** | Pharmacological ascorbate can kill cancer cells by increasing oxidative stress via two possible mechanisms that complement each other. First, extracellular H_2O_2 may directly kill cancer cells by generating •OH via the Fenton reaction[25,26,135]. Increased levels of labile ferric iron, Fe^{3+}, in the tumour microenvironment can facilitate the oxidation of ascorbate, resulting in ascorbate radical, DHA and ferrous iron, Fe^{2+}. Once Fe^{2+} is formed, Fe^{2+} may be oxidized by oxygen, producing superoxide anions, $O_2^{\bullet-}$. Superoxide dismutase (SOD) catalyses the conversion of $O_2^{\bullet-}$ to H_2O_2 and O_2. Fe^{3+} can enter the cell when bound to transferrin (Tf), which binds to the Tf receptor (TfR) and is processed and oxidized in the endosome to then contribute to the intracellular Fe^{2+} pool[36]. H_2O_2 can enter the cell through diffusion facilitated by aquaporins[136]. H_2O_2 reacts with either extracellular or intracellular labile Fe^{2+} to generate highly reactive hydroxyl radicals (•OH) that are harmful to cells. These reactions are further perpetuated by the recycling of Fe^{3+} to Fe^{2+} by ascorbate and ascorbate radical, generating fully oxidized vitamin C, DHA. Second, H_2O_2 may contribute to the increased levels of extracellular DHA by creating a more oxidative tumour microenvironment. DHA can then efficiently enter cells through glucose transporter 1 (GLUT1) and consume the intracellular reducing potential of reduced glutathione (GSH) and NADPH, resulting in increased levels of intracellular ROS[51,52]. This leads to poly(ADP-ribose) polymerase (PARP) activation, a DNA repair enzyme, thereby depleting cellular NAD^+ levels, a cofactor of PARP. NAD^+ is required by glyceraldehyde 3-phosphate dehydrogenase (GADPH) as a cofactor. Consequent inhibition of GAPDH activity inhibits glycolysis in cancer cells, leading to inhibition of ATP production and cell death[47,137,138]. In addition, cellular ROS can also be released from cells, resulting in a positive feedback loop. Because high levels of labile Fe^{2+}, GLUT1 overexpression and addiction to glycolysis frequently occur in many types of cancer cells, certain cancer cells may present all three of these characteristics and those populations might be more sensitive to ascorbate treatment. 1,3BPG, 1,3-bisphosphoglyceric acid; G3P, glyceraldehyde 3-phosphate; G6PD glucose-6-phosphate dehydrogenase; GSSG, glutathione disulfide; PPP, pentose phosphate pathway; SVCT, sodium-dependent vitamin C transporters.

Box 1 | **Transport of vitamin C**

The highest tissue concentrations of vitamin C are found in the brain, the adrenal gland and white blood cells, with concentrations ranging from 1 to 15 mM. These concentrations are 15–200 times higher than those in the plasma owing to active transport mechanisms[4], mainly via sodium-dependent vitamin C transporters (SVCT1 and SVCT2)[115]. SVCT1 is mainly expressed in intestinal and renal epithelial cells, where it mediates absorption and re-absorption of vitamin C, respectively. SVCT2 is expressed throughout the entire body and is considered to be the primary vitamin C transporter. Interestingly, 50% of SVCT1-knockout mice do not survive until weaning, and the deletion of SVCT2 in mice leads to neonatal death[116,117].

Unlike ascorbate, dehydroascorbic acid (DHA) is transported by a class of facilitative glucose transporters (GLUTs)[118]. Among more than 12 different GLUTs, GLUT1 and GLUT3 have a higher affinity for DHA than for glucose[6]. However, under physiological conditions, GLUT transporters are unlikely the dominant path for ascorbate accumulation in most tissues, because glucose levels in the plasma (2–5 mM) are significantly higher than DHA levels (5–10 μM). Red blood cells, and certain cancer cells, do not express SVCTs but transport vitamin C mainly as DHA via GLUT1 (REF.[8]). The rate of DHA uptake via GLUT1 or GLUT3 is at least 10–20 times faster than ascorbate uptake via SVCTs[119]. This difference is because the highly favourable reduction in intracellular DHA to ascorbate drives DHA uptake by cells. Furthermore, local DHA concentrations in body fluids can be higher under pathological conditions, such as cancer, where ROS released from cancer cells can facilitate the oxidation of extracellular ascorbate to DHA[54,55,120]. For example, intestinal tumours harbouring *KRAS* mutations exhibited an increase in intracellular ascorbate level from 100 μM (basal level) to more than 10 mM within 1 hour following intraperitoneal injection of high-dose ascorbate to a mouse model of intestinal tumours ($Apc^{-/-}$;$Kras^{G12D/+}$) compared with tumours without *Kras* mutation ($Apc^{-/-}$)[47]. This drastic increase in intracellular ascorbate level in KRAS mutant tumours can be explained by the selective uptake of DHA via GLUT1 and its subsequent reduction to ascorbate. Tumours from $Apc^{-/-}$;$Kras^{G12D/+}$ mice highly express GLUT1 compared with $APC^{-/-}$ mice, whereas both tumours express low levels of SVCTs.

synthesis, inhibiting ferritin degradation, suppressing iron efflux and enhancing intestinal absorption of iron[10]. Interestingly, vitamin C also functions as a critical cofactor for numerous enzymes by readily donating its electrons to prosthetic metal ions to achieve full enzymatic activity[4]. In general, these enzymes are categorized into two families: copper-containing monooxygenases and Fe^{2+}-dependent and α-ketoglutarate (αKG; also known as 2-oxoglutarate (2OG))-dependent dioxygenases (αKGDDs). αKGDDs are iron-containing enzymes that consume oxygen and αKG as co-substrates while producing CO_2 and succinate. αKGDDs catalyse a wide range of hydroxylation reactions involved in collagen synthesis, hypoxia-inducible factor 1α (HIF1α) stability, carnitine synthesis, the catabolism of tyrosine and the demethylation of protein, DNA and RNA. Thus, vitamin C is responsible for regulating a variety of important biological processes[11].

Anticancer mechanism of vitamin C

Over the past decade, a growing number of studies have demonstrated that millimolar concentrations of pharmacological vitamin C can kill cancer cells in vitro and slow tumour growth in vivo. However, the mechanism by which some cancer cells are sensitive to vitamin C, while normal cells remain resistant, is poorly understood. Given the diversity of processes affected by vitamin C, the mechanistic basis for vitamin C's action could depend on a variety of different factors, including the type of cancer being treated, and the tumour's dependency on particular pathways. Here, we discuss three distinct vulnerabilities in cancer that can be exploited by pharmacological ascorbate.

Targeting redox imbalance

It is generally accepted that cancer cells experience more oxidative stress compared with normal cells owing to an elevated metabolic rate and defective mitochondria[12]. Although ROS can facilitate tumour development by stimulating cell proliferation and promoting genetic instability, excessive ROS can also be detrimental to cancer cells. To compensate, cancer cells often enhance pathways that help mitigate the toxic effects of ROS[13]. On the basis of the premise that ROS promotes cancer development, antioxidant treatment has been investigated as an anticancer strategy. However, recent results from both human and animal studies have found no clear evidence of the benefit of antioxidant treatment in preventing or suppressing cancer development. In some cases, antioxidant treatment even appeared to accelerate cancer progression and metastasis in mouse models of lung adenocarcinoma and melanoma[14–16] and increase the risk of prostate and lung cancers in patients[17–19]. Together, these results indicate that certain cancer types may rely on antioxidants for survival and may thus be vulnerable to pro-oxidant therapies. Indeed, pro-oxidant anticancer therapies, such as radiation, have been employed in the clinic[20]. However, current pro-oxidant strategies often cause serious collateral damage, resulting in a narrow therapeutic window[20]. Here, we propose that pharmacological ascorbate can potentially circumvent this problem by exploring two common features of cancer cells: their increased levels of labile transition metals, especially iron[21], and their increased reliance on glucose uptake and glycolysis[22]. Although we discuss these two mechanisms individually in this section, they are not mutually exclusive and can occur simultaneously, synergizing the selective toxicity of ascorbate in cancer cells (FIG. 1b).

Increased labile iron level. In the presence of redox-active transition metals, such as iron, vitamin C exerts pro-oxidant effects. Iron is an essential prosthetic metal ion for a number of proteins[23]. Most organisms require iron owing to its unique ability to efficiently switch between two oxidation states — ferrous iron (Fe^{2+}, reduced) and ferric iron (Fe^{3+}, oxidized) — in response to changes of ligands in the environment. Because of iron's highly reactive biochemical property, most labile iron must be sequestered by transferrin (Tf) in plasma, stored in ferritin inside the cell or embedded as cofactors, such as haem, in proteins[24]. However, cells also keep small pools (~3–5% or ~1 μM in humans) of loosely coordinated Fe^{2+}, called labile iron pools, in the cytosol and the mitochondrial matrix for easy access[24]. When labile Fe^{2+} in these pools reacts with H_2O_2, it can generate the damaging hydroxyl radical (•OH) via the Fenton reaction[21] (FIG. 1b). To perpetuate this reaction, ascorbate effectively donates electrons to Fe^{3+} to regenerate redox-active Fe^{2+}, thereby generating ROS continuously and contributing to cell death[25]. Thus, although iron is essential for a variety of biological processes, it can be a dangerous liability at the same time.

Numerous in vitro cell culture studies have shown that pharmacological ascorbate produces extracellular H_2O_2, which can directly kill cancer cells[26–28]. However, the exact mechanisms for this observation are currently unclear. Some studies showed that extracellular H_2O_2 can be generated via spontaneous autoxidation even in the absence of iron by reacting with oxygen when supraphysiological, millimolar concentrations of ascorbate are added to the medium in both cell-free and cell culture systems[29,30]. Other studies demonstrated

that labile metals, especially Fe^{2+} in the medium, catalyse ascorbate autoxidation, thereby generating extracellular H_2O_2 in both cell-free and cell culture systems[31,32]. Because most labile Fe^{2+} is known to be sequestered by Tf in vivo[24], these studies argue that ascorbate's ability to produce H_2O_2 is an in vitro artefact[33,34]. Disputing against this hypothesis, it was shown that $Asc^{\bullet-}$ and H_2O_2 were generated in vivo following intravenous ascorbate injections in rats ($0.5\,g\,kg^{-1}$), and the production was ascorbate-dose-dependent[35]. In another study, daily intraperitoneal injection with high-dose ascorbate ($4\,g\,kg^{-1}$) inhibited neuroblastoma growth in a xenograft model, and tumours had the increased activity of checkpoint kinase 2 (CHK2) and histone 2AX (H2AX). This observation suggests that pharmacological ascorbate can cause DNA damage in in vivo tumours, although an in vivo link between ascorbate and the generation of H_2O_2 has not been demonstrated in this study[36].

The important question then is how extracellular H_2O_2 generated from pharmacological ascorbate can contribute to the selective toxicity to cancer cells compared with normal cells. If ascorbate generates extracellular H_2O_2 equally in both cancer cells and normal cells regardless of the exact mechanism (for example, autoxidation and/or liable iron reactions in the medium or the serum in vivo), pharmacological ascorbate would not provide any additional advantage over other pro-oxidant therapies in terms of therapeutic window. Here, we propose three potential mechanisms by which ascorbate-induced H_2O_2 selectively kills certain types of cancer cells compared with normal cells. First, if the tumour microenvironment is enriched for labile iron, its reaction with ascorbate will generate H_2O_2 and $^{\bullet}OH$, which can be lethal to cancer cells. Second, if tumour cells have increased levels of intracellular labile iron compared with normal cells, extracellular H_2O_2 from ascorbate autoxidation can diffuse into tumour cells and react with the intracellular pools of labile iron and generate $^{\bullet}OH$ within tumour cells. Third, extracellular H_2O_2 can contribute to the increased level of extracellular DHA by generating an oxidative microenvironment. As a result, DHA is transported into tumours that express high levels of glucose transporter 1 (GLUT1), generating oxidative stress in those cells, which is discussed in the following section. In this section, we discuss the first and second possibilities.

Numerous iron-containing and haem-containing enzymes are involved in many important cellular processes such as cellular respiration, DNA synthesis, cell cycle and epigenetics[23,36]. For this reason, cancer cells have a high demand for easily accessible labile Fe^{2+} for their survival and growth. It has been shown that reprogramming of iron metabolism can occur via a variety of mechanisms, including the upregulation of several iron-intake pathways or downregulation of iron export and storage pathways in various types of cancer such as breast, prostate and lymphoma[37,38]. For example, the pool of intracellular labile Fe^{2+} in breast cancer cells is approximately twice as high as in normal breast epithelial cells[39]. In addition, tumour-associated macrophages may promote iron release in the tumour microenvironments[40,41]. Patients with advanced breast cancer have significantly higher levels of Fe^{2+} in plasma than healthy human control groups[42]. Thus, tumour cells with the high levels of extracellular and/or intracellular labile iron may be more vulnerable to high-dose ascorbate than normal cells as they can generate more H_2O_2 and $^{\bullet}OH$ than normal cells (FIG. 1b). Supporting this hypothesis, a recent study showed that an increased level of mitochondrial ROS in lung and glioblastoma cancer cells led to increased levels of intracellular labile iron through the upregulation of the Tf receptor (TfR), thus increasing the sensitivity of these cancer cells to ascorbate[43]. Another study indicated that multiple myeloma cells have an elevated labile iron pool owing to the low expression of the iron exporter, ferroportin 1, which led to their selective sensitivity to pharmacological doses of ascorbate[44]. Despite these promising preclinical studies, researchers still need to determine whether there is any correlation between ascorbate sensitivity, ascorbic radical or H_2O_2 production and intracellular labile Fe^{2+} levels in a large set of cancer cell lines or patient samples. Moreover, identifying potential biomarkers or gene expression signatures to predict ascorbate sensitivity related to the increased labile iron level would be useful in the clinic.

Increased DHA uptake via GLUT1. Tumour cells exhibit a high rate of glycolysis, even in conditions with ample oxygen — a phenomenon that was first described by Otto Warburg, nearly a century ago[22]. This metabolic reprogramming, also known as the Warburg effect, is essential for tumour survival and proliferation[45]. Oncogenic *KRAS* or *BRAF* mutations contribute to the Warburg effect, in part by upregulating GLUT1 (REF.[46]). These results suggest that exploiting the selective expression of GLUT1 and the metabolic liability associated with increased reliance on glycolysis may be a viable therapeutic strategy to target cancer. Indeed, we recently showed that high-dose ascorbate can target these vulnerabilities in *KRAS* or *BRAF* mutant colorectal cancer (CRC) cells[47]. When ascorbate is administered, it is oxidized to DHA. Owing to its structural similarity to glucose, DHA is efficiently taken up via GLUT1 in *KRAS* or *BRAF* mutant cells[47,48] (BOX 1). Inside the cell, DHA is rapidly reduced back to ascorbate at the expense of GSH and NADPH[47,49]. This reduction depletes intracellular antioxidants and increases endogenous levels of ROS[47,50]. The elevated ROS, in turn, inactivates a glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), by oxidizing a cysteine residue in the active site. In addition, ROS leads to activation of poly(ADP-ribose) polymerase (PARP), which in turn leads to depletion of NAD^+ (a key cofactor of GAPDH), thereby further inhibiting GAPDH[47] (FIG. 1b). Inhibiting GAPDH in highly glycolytic *KRAS* or *BRAF* mutant cells ultimately leads to an 'energy crisis' and cell death not seen in their wild-type counterparts[47]. Consistent with in vitro results, daily intraperitoneal injection of ascorbate ($4\,g\,kg^{-1}$) inhibited tumour growth in $Apc^{-/-}$;$Kras^{G12D/+}$ mutant mice whereas it did not affect tumour growth in $Apc^{-/-}$ mice[47]. Our findings also suggest that ascorbate therapy may be extended to other cancers as long as they present high GLUT1 expression and high glycolytic activity. For example, recent studies showed that gastric cancers and von Hippel-Lindau (VHL)-null renal cancers, which have high GLUT1 expression and addiction to glycolysis, were selectively killed by high-dose ascorbate, supporting our proposed mechanism[51,52].

Owing to the unstable nature of DHA and the chemical and biological equilibrium between ascorbate and DHA, it is difficult to quantify the exact amount of DHA generated from ascorbate. Despite this challenge, many studies have shown that cancer cells with high GLUT1 but not sodium-dependent vitamin C transporters exclusively take up ascorbate in the form of DHA both in vitro or in vivo[6,8,47,53]. Although our study indicated that DHA is the pharmacologically active agent, it is important to note that ascorbate (not DHA) needs to be used for both preclinical and clinical anticancer therapies. Bolus treatment of high-dose DHA has only transient effects on cancer cells in vitro and in vivo owing to its extreme instability in neutral pH[25]. Moreover, degradation of DHA generates

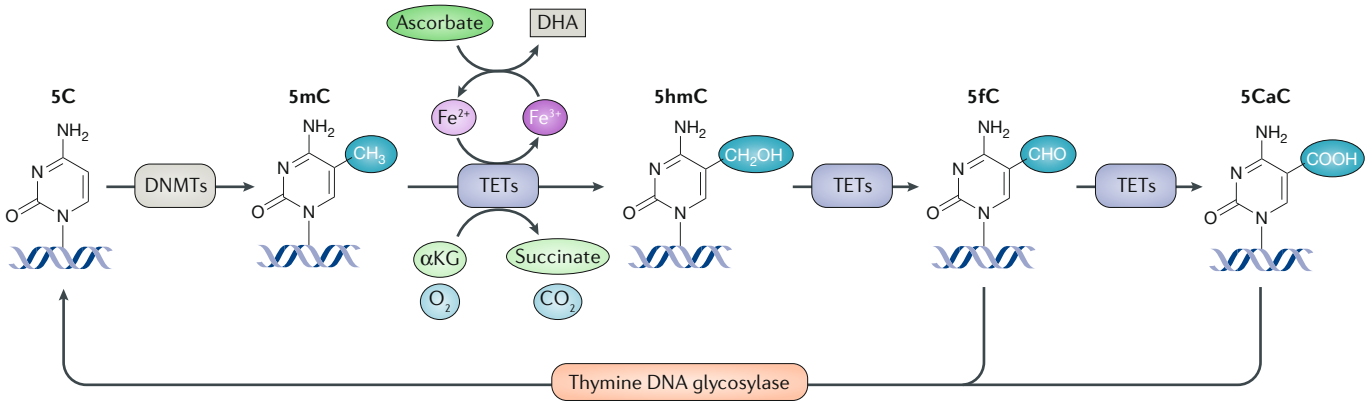

Fig. 2 | **Regulation of TET enzymes by ascorbate.** Catalysed by DNA methyltransferases (DNMTs), DNA methylation occurs at the carbon-5 position of cytosine. Intracellular ascorbate influences the DNA methylation landscape by enhancing the enzymatic activity of ten-eleven translocation enzymes (TETs), which actively remove cytosine methylation marks through a series of oxidation reactions dependent on oxygen, α-ketoglutarate (αKG), Fe^{2+} and ascorbate on the basis of its function as an αKG-dependent dioxygenase (αKGDD)[139,140]. TETs first convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). In the next two steps, 5hmC is further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5CaC). Subsequently, 5fC and 5CaC are converted to cytosine by the base excision repair pathway enzyme, thymine DNA glycosylase. By promoting the recycling of Fe^{3+} to Fe^{2+}, ascorbate ensures that TETs are constantly active. DHA, dehydroascorbic acid.

many undesirable chemicals such as 2,3-DKG and oxalate[25], which may confound the efficacy of ascorbate therapy. By contrast, ascorbate has a significantly longer half-life in cell culture medium and plasma. Because the tumour microenvironment is known to be oxidative[54,55], adding high concentrations of ascorbate would efficiently and continuously generate DHA from ascorbate. Moreover, when the oxidation of millimolar levels of ascorbate generates extracellular H_2O_2 as we discussed before, this will result in high amounts of extracellular DHA as DHA is the main oxidized form of ascorbate.

The effect of oxidative stress in cancer cells due to high-dose ascorbate via the mechanism discussed above is also supported by evidence in humans. Patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency showed haemolytic anaemia following high-dose intravenous ascorbate therapy[56–59]. Although erythrocytes (red blood cells) have a high expression of GLUT1 and are dependent on glycolysis for their energy source (similar to *KRAS* or *BRAF* mutant cells), they have an increased level of antioxidant enzymes and enhanced glucose flux into the pentose phosphate pathway (PPP)[60,61] to generate more NADPH. In a normal setting, this protects erythrocytes from high-dose ascorbate. However, human erythrocytes without G6PD, the rate-limiting enzyme in PPP, cannot produce enough NADPH, a critical molecule for recovering depleted levels of GSH caused by vitamin-C-induced oxidative stress, which leads to the death of erythrocytes, thereby causing anaemia. For this reason, patients who plan to receive intravenous vitamin C therapy should be pre-screened for G6PD deficiency to avoid this complication. The fact that the most obvious and immediate oxidative stress effects of high-dose ascorbate in this genetic disorder occur in erythrocytes indicates the importance of increased GLUT1 levels and dependency on glycolysis in ascorbate-induced oxidative stress.

Targeting epigenetic regulators

Epigenetic reprogramming in cancer includes DNA hypermethylation, which frequently occurs on CpG island promoter regions and is known to silence tumour suppressors such as the retinoblastoma and VHL tumour suppressors[62]. Most of the aberrant DNA hypermethylation patterns observed in cancer can be explained by mutations or altered expression of two protein families: gain of function of DNA methyltransferases (DNMTs; which methylate cytosine to generate 5-methylcytosine (5mC)) and loss of function of ten-eleven translocation (TET) proteins.

TET proteins (TET1, TET2 and TET3) demethylate DNA and belong to the αKGDD enzyme family. Using oxygen and αKG, they catalyse multiple oxidation reactions, first converting 5mC to 5-hydroxymethylcytosine (5hmC), which ultimately results in an unmodified cytosine (FIG. 2). TET2 is frequently mutated or lost somatically in both myeloid and lymphoid malignancies[63,64]. Interestingly, in a large acute myelogenous leukaemia (AML) cohort, TET2 was found to be mutually exclusive with gain-of-function mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 (REF.[65]). IDH1 and IDH2 convert isocitrate to αKG in the cytosol and mitochondria, respectively. However, a neomorphic mutation in IDH1 and/or IDH2 causes a change in enzymatic activity and the accumulation of 2-hydroxyglutarate (2-HG), which inhibits the function of αKG-dependent enzymes such as TET2, resulting in the loss of 5hmC, an increase in DNA methylation and ultimately altered gene expression programmes that drive cancer development[66].

Vitamin C can activate TETs as a cofactor and is required for optimal activity. Ascorbate can donate an electron to Fe^{3+} to generate Fe^{2+}, which is required for TET activity (FIG. 2). The majority of TET2 mutations in AML are heterozygous, and each TET isoform has some functional redundancy. Therefore, ascorbate treatment may enhance the activity of residual TET proteins and thus rescue the abnormal DNA methylation pattern. Indeed, the growing number of recent studies supports this hypothesis. For example, daily intraperitoneal injection of high-dose ascorbate ($4\,g\,kg^{-1}$) treatment in an inducible TET2 deletion mouse model of leukaemia recapitulated the TET2 restoration phenotypes by promoting DNA demethylation and the expression of genes critical for myeloid cell differentiation[67]. Similarly, ascorbate treatment in vitro increased DNA demethylation at enhancers and promoters of genes associated with myeloid differentiation and increased the expression of several key haematopoietic genes in murine bone marrow cells expressing mutant IDH1 (REF.[68]). In certain types of lymphomas where TETs are frequently mutated, ascorbate treatment

Box 2 | **Oral intake of high-dose vitamin C and cancer prevention**

Patients with cancer often have lower plasma concentrations of ascorbate than healthy adults[121,122], and vitamin C deficiency is associated with an increased risk of cancer mortality[123,124]. In Western countries, plasma ascorbate levels can vary among individuals, but the lowest quartile of men have a significantly higher risk of mortality from cancer[125]. In a meta-analysis of 21 studies, including ~9,000 lung cancer cases[126], a correlation was shown between an individual's risk of lung cancer and their vitamin C intake, where male adults in the USA who took 100 mg per day of dietary vitamin C had a 7% reduced lung cancer risk. This dose is also associated with a reduced overall mortality and breast-cancer-specific mortality in women[127,128]. That vitamin C may prevent or delay cancer development was further supported by preclinical studies. In ascorbate-deficient *Gulo*$^{-/-}$ mice, ten-eleven translocation 2 (TET2) deletion drives development of acute myelogenous leukaemia (AML)[72], which is suppressed by administration of oral ascorbate. Similarly, administration of oral ascorbate 7 days before inoculation of cancer cells decreased tumour development in a lymphoma xenograft model[79]. Taken together, oral doses of vitamin C may be an effective agent to prevent the development of certain types of malignancy, especially for individuals who may have mutations such as TETs predisposing them to cancer.

Genetic variation in ascorbate transporters has also been found to associate with cancer risk[129–131]. Although the correlation with cancer remains weak and plasma ascorbate was often not measured, single-nucleotide polymorphisms (SNPs) in the sodium-dependent vitamin C transporters *SLC23A1* (SVCT1) and *SLC23A2* (SVCT2) have been linked to the risk of certain cancers[132,133]. Some of these SNPs are associated with reduced levels of ascorbate systemically, which may have adverse consequences for ascorbate-dependent biochemical processes that involve, for example, Fe^{2+}-dependent and α-ketoglutarate-dependent dioxygenases[134]. However, it remains unclear whether the reduction in ascorbate levels associated with those SNPs can be directly attributed to compromised function of transporters. Together, these findings support the profound importance of the amount of oral ascorbate to humans' optimized health and disease prevention. However, to address the definite benefits of supplementary oral intake of ascorbate, potentially in combination with therapeutic intravenous ascorbate therapy, rigorous preclinical and randomized clinical trials will be required.

in vitro increased TET activity, leading to DNA demethylation, increased expression of tumour suppressor genes and increased chemosensitivity[69]. Of note, all these studies applied appropriate controls to exclude oxidative stress in response to high-dose ascorbate as a possible mechanism by adding catalase (which converts H_2O_2 to water) to culture media, monitoring changes in cellular ROS levels and/or using 2-phosphate L-ascorbic acid, a vitamin C derivative that is stable and not oxidized in typical culture conditions, in their experiments. In addition to blood cancers, ascorbate treatment in melanoma and bladder cancer cells also enhanced 5hmC levels and decreased their malignancy[70,71], suggesting that ascorbate treatment may also be effective in solid cancer with low levels of 5hmC. Taken together, the results from these preclinical models warrant further investigation in the utilization of vitamin C therapy in patients with cancer who present with decreased levels of 5hmC and/or decreased TET activity.

A recent study also suggests that oral ascorbate may have a preventive role in leukaemogenesis[72]. Using several different genetically engineered mouse models (GEMMs) including *Gulo*$^{-/-}$ mice, which are unable to produce vitamin C, and *Tet2*$^{-/-}$ mice, ascorbate deficiency was shown to dysregulate HSC function in both a TET2-dependent and TET2-independent manner, leading to leukaemogenesis. On the basis of this study and the observational human studies (BOX 2), it would be important to determine the adequate amount of oral ascorbate to optimize human health and prevent chronic diseases such as cancer.

In addition to TETs, other αKGDDs such as JmjC domain-containing histone demethylase family (JHDM) and αKGDD AlkB (ALKB) are also known to be epigenetic regulators that can be potentially regulated by vitamin C[73,74]. However, it is currently unclear whether these enzymes have important roles in cancer development and whether ascorbate availability influences their activity, thereby affecting the growth and survival of cancer cells.

Targeting HIF1 signalling

Many solid tumours encounter hypoxia as tumour masses can obstruct and compress surrounding blood vessels and tumour cells can outgrow new blood vessels. To adapt to this hypoxic microenvironment, tumour cells activate the evolutionarily conserved transcription factor HIF1, leading to activation of a wide range of genes and response programmes that facilitate increased survival in such conditions[75].

HIF1, an important target in cancer therapy, is a heterodimeric transcription factor, consisting of two subunits, the O_2-regulated HIF1α and a constitutively expressed HIF1β (REF.[75]). The key mechanism by which O_2 regulates HIF1α activity is through proline hydroxylase domain proteins (PHD1, PHD2 and PHD3) and asparagine hydroxylase (factor-inhibiting HIF (FIH)), collectively known as the HIF hydroxylases. Under normoxic conditions, PHDs hydroxylate proline residues on HIF1α. Prolyl-hydroxylated HIF1α is then bound by the VHL tumour suppressor protein, which recruits an E3-ubiquitin ligase that targets HIF1α for proteasomal degradation (FIG. 3). On the other hand, FIHs hydroxylate an asparagine residue on HIF1α, which blocks the association of HIF1α with the p300 co-activator protein, resulting in the inhibition of the transcription activity of HIF1 (FIG. 3). Similar to TETs, both HIF hydroxylases belong to Fe^{2+}-containing αKGDDs that require O_2 and αKG as substrates. Because of their relatively lower affinity for O_2 (Michaelis constant (K_m) = 230–250 μM) compared with other αKGDDs[76], under hypoxic conditions, PHDs and FIHs are inactive, leading to the stabilization and activation of HIF1. Similar to TETs, HIF hydroxylases require ascorbate as a cofactor to recycle Fe^{2+}. Therefore, cells deficient in ascorbate can have increased HIF1α function, potentially contributing to tumour progression. This implies that ascorbate treatment may enhance the activity of HIF hydroxylases, thus inhibiting HIF1α activity and suppressing tumour growth[77]. Supporting this notion, there is growing evidence that HIF1α-dependent tumour growth may be inhibited by ascorbate[78,79]. Ascorbate level was inversely correlated with HIF1α expression in thyroid lesions[80], and in vitro study showed that ascorbate treatment induced a dose-dependent decrease in expression of HIF1α and GLUT1 (a downstream target of HIF1) in thyroid cancer cells[80]. In studies with *Gulo*$^{-/-}$ mice, lung carcinoma implanted in *Gulo*$^{-/-}$ mice grew slowly when mice were treated with high-dose ascorbate either in drinking water (3.3 g per litre) or daily intraperitoneal injection (1 g kg^{-1}) as compared with *Gulo*$^{-/-}$ mice treated with low-dose ascorbate in drinking water (0.33 g per litre), and tumours exposed to high-dose ascorbate also had reduced expression levels of HIF1α, VEGF and reduced microvessel density compared with control mice[81–83]. In addition, retrospective human studies also support the connection between vitamin C, HIF1 activity and tumorigenesis. Using human patient tumour samples and paired controls for endometrial cancer, renal cell carcinoma (RCC) and CRC, it was

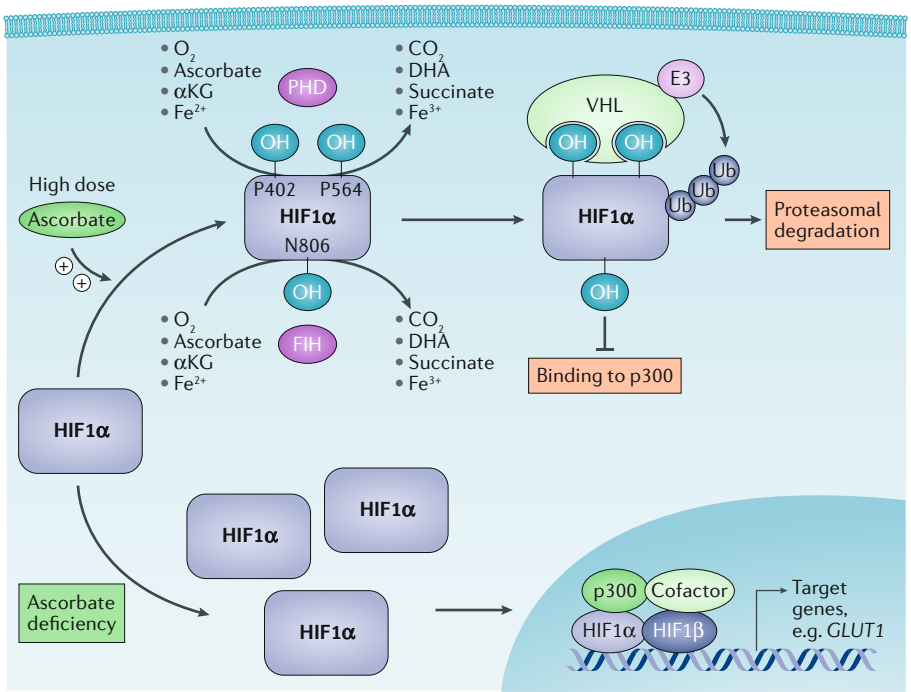

Fig. 3 | **Ascorbate and HIF1α regulation.** Ascorbate is a vital cofactor for the hypoxia-inducible factor (HIF) hydroxylases, proline hydroxylase domain proteins (PHDs) and asparagine hydroxylase (factor-inhibiting HIF (FIH)), which are also members of the α-ketoglutarate (αKG)-dependent dioxygenase (αKGDD) protein family[77,141]. HIF1, a heterodimeric transcription factor, consists of two subunits: HIF1α, regulated by O_2, and HIF1β. Under normal conditions with sufficient oxygen and ascorbate availability, the functional capacity of HIF1α is inhibited by the HIF hydroxylases. HIF1α is hydroxylated at proline residues by PHD. Prolyl-hydroxylated HIF1α is then bound by the von Hippel-Lindau (VHL) tumour suppressor protein, which recruits an E3-ubiquitin ligase that targets HIF1α for proteasomal degradation and thus limits the quantity of HIF1α units within the cell. HIF1α activity is regulated within the nucleus and can be inhibited. FIH hydroxylates an asparagine residue, N806, on HIF1α. This hydroxyl group prevents p300, a co-activator protein, from associating with the HIF complex, resulting in the inhibition of the transcription activity of HIF1 and the activation of any downstream pathways. High-dose ascorbate treatment in tumour tissues with normoxic HIF1α stabilization can potentially increase PDH and FIH activity to degrade HIF1 protein and slow down tumour growth. In conditions where ascorbate is depleted, such as in certain cancer types or in tumour tissue in *Gulo*$^{-/-}$ mice, the activity of PDH and FIH is reduced even when oxygen is available, which leads to stabilization and activation of HIF1α and its translocation to the nucleus. HIF1α associates with HIF1β, p300 and other cofactors within the nucleus to induce target genes such as *GLUT1*, which together might promote tumour growth.

demonstrated that tumours that had the highest HIF1 activity were those deficient in ascorbate in tumours[84–86]. It was also shown that patients with CRC who had high levels of ascorbate in their tumour had a better patient outcome and longer patient survival after surgery[84]. Taken together, these data suggest that high-dose ascorbate treatment can slow tumour growth by moderating HIF1α. However, all studies thus far prove the association but not the causality. It would be interesting to see whether the deletion of PHDs or FIHs in tumour-bearing *Gulo*$^{-/-}$ mice abolishes the effects of high-dose ascorbate.

The stabilization of HIF1α even under normoxic conditions can occur, for example, in RCC, where deletion of the VHL tumour suppressor prevents HIF1α degradation in normoxia. VHL-deficient RCC cells undergo cell death when exposed to vitamin C in normoxia, in contrast to isogenic VHL-proficient cells[52]. Mechanistically, the higher levels of GLUT1, a HIF1α downstream target, in normoxic VHL-deficient cells than in VHL-proficient cells facilitated increased uptake of ascorbate-derived DHA, leading to increased generation of ROS and cell death. In line with this, GLUT1 knockdown in VHL-deficient RCC cells conferred resistance to vitamin-C-induced toxicity. In addition to VHL mutations, HIF1α stability and activity can also be increased by mutations of two tumour suppressor enzymes in the tricarboxylic acid (TCA) cycle: succinate dehydrogenase (SDH) and fumarate dehydrogenase (FH). Loss-of-function mutations in SDH and FH cause a build-up of succinate and fumarate, respectively[87]. Accordingly, increased levels of succinate and fumarate caused by mutations in these genes can compete with αKG and inhibit activity of HIF hydroxylases and thus induce normoxic HIF1α activity in vitro[88,89]. Because inherited or somatic mutations in SDH and FH are tightly associated with the development of several tumours, such as paraganglioma, pheochromocytoma and RCC[90–92], it would be interesting to investigate whether ascorbate treatment of these mutant cells leads to inhibition of HIF1 activity and decreased malignancy in vivo.

Vitamin C as anticancer therapy

High-dose vitamin C should not be viewed as a 'one-size-fits-all' modality for cancer treatment. Understanding the critical differences between oral and intravenous ascorbate administration routes and knowing which patients to treat will be critical for clinical trial designs and the approval of new ascorbate therapies. Clinical trials in the 1970s involving patients with terminal cancer showed that intravenous high-dose ascorbate extended patient survival in response to ascorbate treatment[93,94], whereas large-scale randomized controlled trials (RCTs) in the 1980s failed to confirm these initial findings because ascorbate was given orally instead[95,96]. As it turns out, the differences between oral and intravenous administration routes can affect the maximum achievable plasma concentration in patients. In the initial trials, ascorbate was administered both intravenously and orally and achieved a peak plasma concentration of 6 mM. However, in later trials, where the same dose of ascorbate was administered orally, a peak plasma concentration of less than 200 μM was achieved[97]. It is now widely accepted that the millimolar concentration of ascorbate needed to induce cytotoxicity in cancer cells can be achieved only when administered intravenously[97]. For example, a phase I clinical study revealed that ascorbate concentrations could safely reach 25–30 mM with intravenous infusion of 100 g of vitamin C[98]. In this study, plasma concentrations around 10 mM were sustained for at least 4 hours, which, on the basis of preclinical studies, is sufficient to slow the growth of cancer cells.

An increased understanding of the clinical pharmacokinetics of ascorbate has provided confidence in revisiting the clinical potential of ascorbate. Consequently, over the past decade, there have been an increased number of phase I/II clinical

trials and case reports testing the safety and efficacy of high-dose ascorbate as a treatment for various cancer types such as ovarian, brain, prostate and lung cancers as a monotherapy or in combination with radiation and other conventional chemotherapies[3,99,100]. In short, a significant number of clinical studies to date have indicated that intravenous high-dose ascorbate is well tolerated in patients with minimal toxicity, improves the quality of life for patients and has demonstrated synergistic therapeutic effects as well as reduced side effects when combined with radiation and standard chemotherapies. However, many of these studies were not designed as large-scale RCTs; thus, the efficacy of high-dose ascorbate therapy remains to be determined. Below, we discuss the future direction of clinical research into ascorbate as a cancer therapy.

Glossary

2-Phosphate l-ascorbic acid
A derivative of ascorbate that is not oxidized in culture or serum but releases ascorbate once it is inside the cells via hydrolysis mediated by alkaline phosphatase on the plasma membrane.

5-Aza-CdR (decitabine)
A cytidine antimetabolite analogue that incorporates into DNA and inhibits DNA methyltransferase (DNMT) activity, which results in DNA demethylation (hypomethylation).

Biomarkers
Any biological measurable indicators of the severity or presence of some disease state.

Fenton reaction
A chemical reaction that converts hydrogen peroxide into a highly toxic hydroxyl radical in the presence of labile iron.

Ferritin
A protein that contains iron and is the primary form of iron stored inside of cells.

Free radicals
Molecules possessing unpaired electrons and thus are reactive and short-lived in a biological setting.

Haem
An iron-containing group that gives myoglobin and haemoglobin the ability to bind oxygen.

Hydroxyl radical
(•OH). A highly reactive and short-lived radical that attacks any molecule in its immediate vicinity, especially DNA, protein and lipids, eventually leading to cell death.

Imatinib
A BCR-ABL-selective tyrosine kinase inhibitor, also known as Gleevec. Imatinib has been used to treat chronic myelogenous leukaemia and acute lymphocytic leukaemia.

Intraperitoneal (IP) injection
Giving medicines or fluids into the peritoneum (body cavity), which is more often applied to animals than to humans.

Intravenous injection
Giving medicines or fluids through a needle or tube inserted into a vein, allowing them to enter the bloodstream immediately.

Michaelis constant
(K_m). The substrate concentration at the half of the maximum velocity (V_{max}). An enzyme with a high K_m has a low affinity for its substrate and requires a greater concentration of substrate to achieve V_{max}.

Parenteral injection
Giving medicine or fluids intravenously (into a vein), subcutaneously (under the skin) and intraperitoneally (into the peritoneum).

Pharmacodynamics
The study of the biochemical and physiological effects of drugs. Generally refers to the dose–response relationship for a particular drug.

Pharmacokinetics
The activity of drugs in the body over a period, including the processes by which drugs are absorbed, distributed in the body, localized in the tissues and excreted.

Pharmacological ascorbate
Intravenous or intraperitoneal delivery of vitamin C, which allows for plasma concentrations to reach the millimolar scale.

Physiological ascorbate
An oral dose of dietary vitamin C, usually resulting in a peak plasma concentration of 200 μM.

Predictive biomarkers
A biomarker that gives information about the effect of a therapeutic intervention.

Prosthetic
A group that is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. It may be organic or inorganic (such as a metal ion), but not amino acids.

Randomized controlled trials
(RCTs). A study design that randomly assigns participants into an experimental group or a control group (or placebo group).

Reactive oxygen species
(ROS). Derivatives of oxygen that are more reactive than molecular oxygen.

Single-nucleotide polymorphisms
(SNPs). A variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some high degree within a population (for example, >1%).

Therapeutic window
The range of doses of a drug that can treat disease effectively without having toxic effects.

Transferrin
(Tf). The main protein in the blood that binds to iron and transports it throughout the body.

Vemurafenib
A selective V600E mutant BRAF kinase inhibitor, also known as PLX4032. It has been used to treat BRAF V600E mutant melanoma.

From bench to bedside

In this Review, we propose that vitamin C serves as an excellent example of a natural compound that targets multiple vulnerable nodes to inhibit cancer growth. Although we identified three distinct mechanisms by which high-dose ascorbate can inhibit cancer growth, it is possible that each mechanism may not work independently. For example, subpopulations of *KRAS* or *BRAF* mutant CRC cells that were not completely killed via the pro-oxidant mechanism in vivo — potentially owing to their distance from blood vessels, poor perfusion[101] or resistance to ROS — may still be affected by ascorbate therapy via inhibition of HIF1 signalling and/or activation of TET enzymes. In fact, reports show that many CRCs have low expression levels of TETs and relatively high expression levels of HIF1α, implying that pharmacological ascorbate may attack multiple nodes in tumours with minimum toxicity[102,103], making it an ideal 'magic bullet' for cancer therapy.

Understanding the mechanisms of action gives us critical information about the patient populations who may receive the most benefit from ascorbate therapy. On the basis of current preclinical studies, ascorbate may be more effective in patients with cancer with mutations in *KRAS, BRAF, TET2, IDH1* and/or *IDH2, VHL, SH* or *FDH*. Pending validation, these could be used as putative predictive biomarkers for ascorbate therapy in clinical trials. Multiple omics assays such as genomics, transcriptomics, proteomics and metabolomics can be performed using a patient's tumour biopsy samples not only to test existing hypotheses but also to generate hypotheses in an unbiased and comprehensive way. Furthermore, a patient's urine, blood and stool samples can also be analysed for omics assays and compared before and after receiving ascorbate treatment. After treatment with pharmacological ascorbate, correlations can be made between the response and disease progression to discover prospective biomarkers and better define the pharmacodynamics of ascorbate. Once clinicians confirm, or identify, promising biomarkers in these early trials, they will be better able to design and optimize a large-scale RCT for ascorbate therapy using an appropriate patient population. Poorly designed clinical trials in the past have stunted critical research on therapeutic

efficacy of ascorbate as a cancer treatment. We must learn from our mistakes and design more thorough trials if we hope to reach a conclusion regarding the benefits of ascorbate in cancer therapy. Moreover, although not discussed extensively in this Review, it would be interesting to investigate the potential benefit of oral vitamin C supplements to prevent cancer (BOX 2).

Combination therapy

Although pharmacological ascorbate alone has been shown to reduce the tumour growth in many different mouse models of cancer, the clinical potential of ascorbate as an anticancer therapy may also lie in its combined use with other cancer therapies[3,11]. Numerous preclinical and clinical studies indicate that parenteral injection of pharmacological ascorbate does not interfere with chemotherapy or radiotherapy and may even act synergistically[43,104,105]. Moreover, because the mechanisms of action of vitamin C are becoming better defined, we can propose vitamin C combinations in a more rational, hypothesis-driven manner. For example, on the basis of ascorbate's known DNA demethylation effects via TET activation, a recent study found that vitamin C enhanced the effect of the DNMT inhibitor 5-aza-CdR (decitabine) by promoting the demethylation of human endogenous retroviruses and amplifying the expression of interferon-stimulated genes, leading to cancer cell death[106,107]. Because decitabine is approved by the US Food and Drug Administration for the treatment of patients with myelodysplastic syndromes, where *TET2* is also frequently deleted[108], it would be interesting to test these combinations in clinical trials.

Combining vitamin C with immune checkpoint inhibitors and immunomodulatory agents, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4) receptor and programmed death 1 (PD-1)–programmed death ligand 1 (PD-L1) blockers, may also lead to durable therapeutic responses against a broad spectrum of cancers. A compelling hypothesis is offered by the utilization and combination of high-dose vitamin C, which is known to enhance the function of both innate and adaptive immune cells[109,110]. Tumours exist in a complex immune milieu that includes neutrophils, macrophages and lymphocytes. Although not much is known about how ascorbate is utilized by the various cells that make up the tumour microenvironment, studies have shown that phagocytes and lymphocytes have ascorbate concentrations 10–100 times greater than plasma[4]. Moreover, a wealth of knowledge is emerging that highlights the influence of ascorbate in inflammatory response and immune cell function[110,111], which suggests that ascorbate may have synergistic effects when combined with current immunotherapy.

Concluding remarks

Despite the unprecedented popularity of ascorbate among the public as an anecdotal 'cure-all' remedy, much of its biological functions and pharmacological activity have remained elusive. However, recent discoveries about the diverse biological functions of ascorbate, and its relevance to cancer therapy, generated exciting and promising hypotheses regarding the use of ascorbate in the treatment of cancer. We have discussed the critical role of ascorbate in the function of TETs, PHDs and FIHs, which are all αKGDDs. Given that more than forty αKGDDs exist[112], it is possible that other αKGDDs could contribute to the anticancer mechanisms of ascorbate. Discovering novel roles of ascorbate and the pathways it regulates will aid in the identification of molecules that can be targeted to sensitize tumours to ascorbate treatment and lead to the development of novel combination therapies.

To fully elucidate the biological functions of ascorbate and its relevance to cancer development, researchers will need to utilize better in vivo models that recapitulate the human condition. GEMMs are powerful tools for studying the pathogenesis of cancer and examining the systemic effects of vitamin C in vivo[113]. Recent studies utilizing GEMMs have enhanced our understanding of anticancer properties of ascorbate and reinforced our understanding of its mechanism of action as it pertains to cancer[47,67,72]. Also, *Gulo*$^{-/-}$ mice are an ideal candidate for vitamin C research as they, similar to humans, cannot synthesize viamin C de novo in the liver. A significant number of human studies consistently show that ascorbate treatment improved quality of life for patients with cancer, and in combination therapy, ascorbate protected normal tissues from toxicity caused by chemotherapy[114]. These effects are likely caused by non-cell autonomous mechanisms, and if so, whole organisms or co-culture systems, rather than cancer cell lines themselves, would be crucial to discovering mechanisms of ascorbate's indirect effects on cancer cells.

In conclusion, high-dose intravenous ascorbate represents a promising and inexpensive anticancer therapeutic option that should be further explored in clinical trials. Given its low toxicity and low financial cost, ascorbate could become an important weapon in our arsenal against cancer, either acting as a single agent with predictive biomarkers or used in combination as an adjuvant therapy. Although we are still waiting on a definitive answer for the clinical benefits of ascorbate therapy in cancer, current preclinical and early phase I/II clinical trial results suggest that Linus Pauling's claims regarding the therapeutic benefits of vitamin C therapy in cancer may not be so outrageous after all.

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