Research Article

# Ascorbic Acid Therapy in Hematological Malignancies - The Current Knowledge and Future Directions

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**Ascorbate therapy in cancer treatment has been highly controversial. Recent data, however, has shed light on many newly recognized functions of ascorbate in the body that could impact cancer cell growth. There is also more knowledge of the pharmacokinetics properties and anti-cancer effects of ascorbate, leading to a flare of research interest. On the other hand, there has been more understanding of the pathogenesis of hematological malignancies with next-generation sequencing. Hematological malignancies are particularly interesting and relevant to ascorbate treatment due to their reliance on epigenetic regulations to control cell differentiation.**

**Ascorbate, in both oral and intravenous formulations, has multiple potential benefits in the treatment of hematological malignancies through its multitargeting effects such as selective cytotoxicity as pro-oxidant, metabolic alteration and inhibition of cancer energy metabolism, epigenetic regulation via the IDH1/2-TET2-WT1 pathway, targeting PML/RARA in acute promyelocytic leukemia and FLT3-ITD in acute myeloid leukemia, regulating hypoxia-inducible factor hydroxylases and other α-ketoglutarate-dependent dioxygenases, immunomodulatory effects via multiple mechanisms including IDO and TDO inhibition, correction of vitamin C deficiencies common in hematological malignancies, and producing synergistic effects with numerous chemotherapeutic agents and targeted therapies. There are proven benefits of adding ascorbate in some of the treatments of hematological malignancies. However, the potential risks of ascorbate should also be considered, including oxidative hemolysis, calcium oxalate stones and oxalate nephropathy, pseudohyperglycemia, and potential inhibitions of other cancer treatments such as boronate proteasome inhibitors. Future clinical trials should be designed with regard to the pharmacokinetics and pharmacodynamics of ascorbate to maximize its safety and benefits.**

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## *List of Abbreviations*

AITL - angioimmunoblastic T-cell lymphoma

AKI - acute kidney injury

AML - acute myeloid leukemia

APL - acute promyelocytic leukemia

ATO - arsenic trioxide

ATRA - all-trans-retinoic acid

$AscH_2$ - ascorbic acid

$AscH^-$ - ascorbate

$AscH^\bullet$ - ascorbyl radical

CAM - complementary and alternative medicine

CLL - chronic lymphocytic leukemia

CMML - chronic myelomonocytic leukemia

CR - complete remission

DHA - dehydroascorbate

DLBCL - diffuse large B-cell lymphoma

DNMTi - DNA methyltransferase inhibitor

DUOX - dual oxidase

EFS - event-free survival

$Fe^{2+}$ - ferrous iron

$Fe^{3+}$ - ferric iron

FLT3 - Fms-like tyrosine kinase 3

FLT3-ITD - FLT3 internal tandem duplication

GAPDH - glyceraldehyde 3-phosphate dehydrogenase

GSH - reduced glutathione

GSSG - oxidized glutathione

Gulo - gulono-gamma-lactone oxidase

G6PD - glucose-6-phosphate dehydrogenase

HIF - hypoxia-inducible factor

HK - hexokinase

HSC - hematopoietic stem cell

$H_2O_2$ - hydrogen peroxide

$IC_{50}$ - 50% inhibitory concentration

IDH - isocitrate dehydrogenase

IDO - indoleamine 2,3-dioxygenase

IV - intravenous

JHDM - jumonji C domain-containing histone demethylases

LFS - leukemia-free survival

MDS - myelodysplastic syndrome

MPPs - multipotent progenitors

$NAD^+$ - nicotinamide adenine dinucleotide

NADPH - nicotinamide adenine dinucleotide phosphate

NOX - NADPH oxidase

ON - oxalate nephropathy

OS - overall survival

PARP - poly ADP ribose polymerase

PML - promyelocytic leukemia

PML/RARA - promyelocytic leukemia/retinoic acid receptor alpha

PTCL-NOS - peripheral T-cell lymphoma, not otherwise specified

QLQ-C30 - European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire

QoL - quality of life

ROS - reactive oxygen species

TCA - tricarboxylic acid

TDO - tryptophan 2,3-dioxygenase

TET - ten-eleven translocation methylcytosine dioxygenase

WT1 - Wilm's tumor 1

αKGDD - α-ketoglutarate-dependent dioxygenases

5-hmC - 5-hydroxymethylcytosine

5-mC - 5-methylcytosine

## Background

Vitamin C, also known as ascorbic acid ($AscH_2$) or ascorbate ($AscH^-$), is an essential water-soluble vitamin that possesses antioxidant and enzyme cofactor activity. Ascorbate acts as a cofactor and antioxidant in many crucial biological processes such as norepinephrine synthesis, collagen hydroxylation, hypoxia-inducible factor (HIF) hydroxylation, and promotion of iron absorption[1]. There is a long history of controversy in utilizing ascorbate as a therapeutic agent for cancer after the earliest reports of cancer patients benefiting from high-dose intravenous (IV) ascorbate about 50 years ago[2][3]. However, the subsequent double-blinded randomized control trial by Mayo clinic showed no effect of high-dose oral ascorbate supplementation versus placebo in patients with advanced cancers, which dampened the research interest in ascorbate[4].

Yet, the later pharmacokinetics studies of ascorbate showed a remarkable difference in plasma concentrations of ascorbate between oral versus intravenous administration. Plasma ascorbate concentration cannot exceed 250 μM even with high-dose oral ascorbate, while plasma ascorbate concentration can reach a level over 15 mM by infusing high-dose IV ascorbate[5][6]. The expression of sodium vitamin C cotransporter 1 in intestinal cells, encoded by SVCT1, would decrease upon exposure to an elevated ascorbate level[7]. The reduction of SVCT1 expression upon exposure to ascorbate probably explained the limited bioavailability of oral ascorbate at higher doses. Together with the results of preclinical studies on the effects of ascorbate on cancer cells, there is a flare of research interest in ascorbate as a potential cancer treatment again.

Various biological and molecular features of hematological malignancies suggest the potential benefits of adding ascorbate to the therapy for these diseases. There are also examples of success in using ascorbate, either in oral or intravenous forms, as part of the therapy of hematological malignancies. Yet, a thorough review of ascorbate in hematological malignancies is lacking. In this article, we review the potential of

ascorbate therapy in hematological malignancies based on the biochemistry of ascorbate, the current evidence on the potential mechanisms of action, potential mechanisms of resistance, safety and toxicity profile, and interaction with other treatments. We would discuss the unresolved questions and the future research directions in the field.

## 1. Biochemistry of ascorbic acid

Ascorbate is a water-soluble vitamin that is important in various biological processes. Szent-Gyorgyi first isolated ascorbic acid in 1923 and was awarded the Nobel prize for this discovery. Ascorbic acid was later synthesized by Haworth and Hirst[8]. Most plants and animals can synthesize ascorbate from glucose or galactose through the uronic acid pathway (Figure 1)[9][10]. Humans cannot synthesize ascorbate due to the evolutionary loss of function of the enzyme L-gulono-γ-lactone oxidase (Gulo), which catalyzes one of the final steps in vertebrate ascorbate biosynthesis[11]. The functional loss of Gulo is common among species with an ascorbate-rich diet, in which the mutation does not pose a selective disadvantage in general[12].

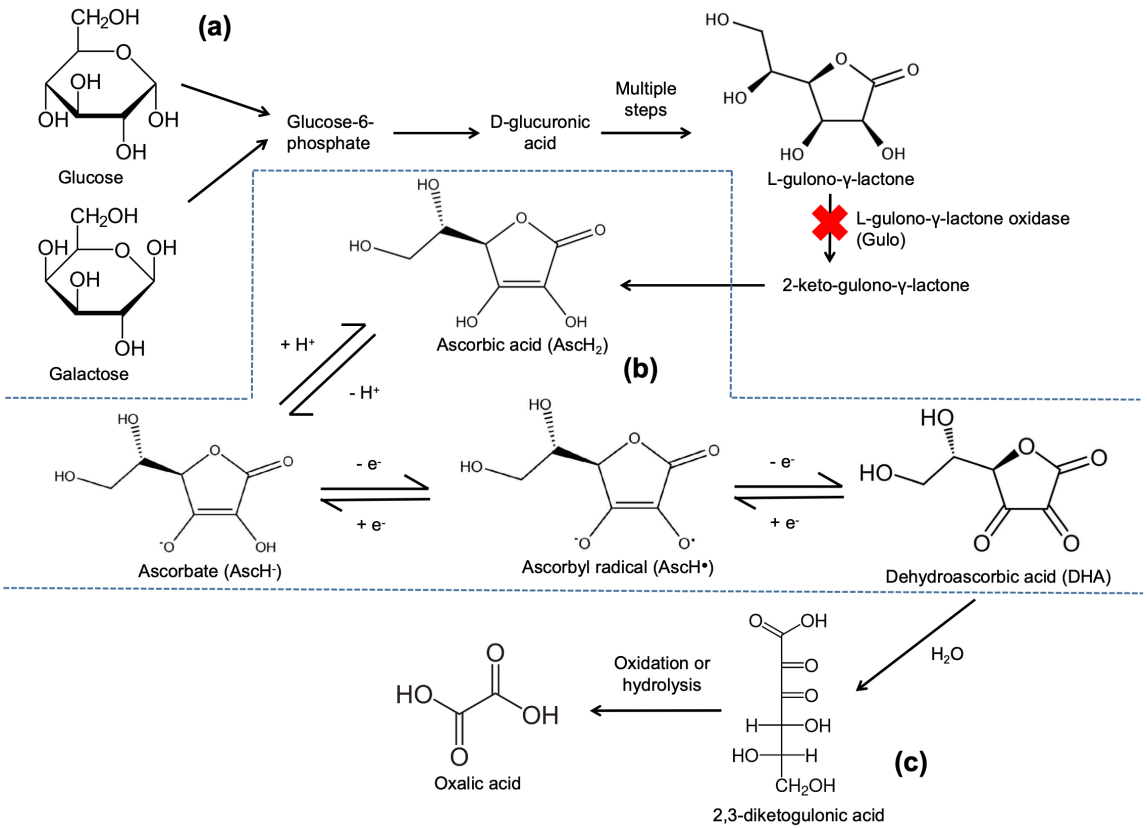


**Figure 1.** The metabolism of ascorbic acid. (a) Biosynthesis of ascorbic acid. Ascorbic acid is synthesized from glucose or galactose via the uronic acid pathway. Humans cannot synthesize ascorbic acid due to the evolutionary loss of L-gulono-γ-lactone oxidase (red cross). (b) Active forms of ascorbic acid. Ascorbic acid would lose a proton to form ascorbate ($AscH^-$), the major form in blood pH. Ascorbate could act as reducing agent by oxidation to ascorbyl radicals ($AscH^•$), which can undergo a disproportionation reaction to form ascorbate and dehydroascorbate. (c) Degradation of ascorbic acid. Dehydroascorbate could be degraded to oxalate by hydrolysis and oxidation.

In circulation, ascorbic acid ($AscH_2$) would lose a proton to form ascorbate ($AscH^-$). Ascorbate acts as a reducing agent, which could be oxidized to ascorbyl radical ($AscH^•$) in redox reactions[13]. Two ascorbyl radicals would undergo a disproportionation reaction to form $AscH^-$ and dehydroascorbate (DHA)[14]. DHA, the fully oxidized form of ascorbic acid, could be reduced to $AscH^-$ by reduced glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) [13]. On the other hand, DHA could be degraded to oxalate by further hydrolysis and oxidation reactions[10].

Ascorbate could enter most cells via SVCTs 1 and 2, encoded by SLC23A1 and SLC23A2 genes[15]. Hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) have higher SLC23A2 expression

than restricted hematopoietic progenitors. On the other hand, DHA enters cells rapidly via glucose transporters, mainly GLUT1 and GLUT3[13][16]. Intracellular DHA could be reduced by GSH, producing ascorbate and oxidized glutathione (GSSG)[17]. GSSG is then reduced back to GSH by glutathione reductase using NADPH.

Ascorbate was demonstrated to react with singlet oxygen and proton to generate $H_2O_2$ and DHA at a very fast rate ($k_q$ = 3.2 x $10^8$ $M^{-1}s^{-1}$)[18]. Ascorbate could also act as a reducing agent to reduce ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$). In the presence of hydrogen peroxide ($H_2O_2$) with $Fe^{2+}$ or $Fe^{3+}$, ascorbate could accelerate the formation of hydroxyl radicals from the Fenton reaction[19]. Although ascorbate acts as a reducing agent, ascorbate is involved in redox reactions which could paradoxically generate reactive oxygen species (ROS) such as $H_2O_2$ and hydroxyl radicals (Figure 2). However, in physiological conditions, the effects of these reactions could be difficult to be appreciated due to the low plasma concentration of ascorbate present (normal serum ascorbate concentration: 26-84 μM)[20].

(a)

$AscH^- + H^+ + O_2 \rightarrow H_2O_2 + DHA \quad k_q = 3.2 \times 10^8\ M^{-1}s^{-1}$ (1)

$AscH^- + Fe^{3+} \rightarrow AscH^\bullet + Fe^{2+}$ (2)

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\bullet + OH^-$ (3)

$AscH^- + H_2O_2 \rightarrow AscH^\bullet + OH^\bullet + OH^-$ (2) + (3) = (4)

$2\ AscH^\bullet \rightarrow AscH^- + DHA$ (5)

(b)

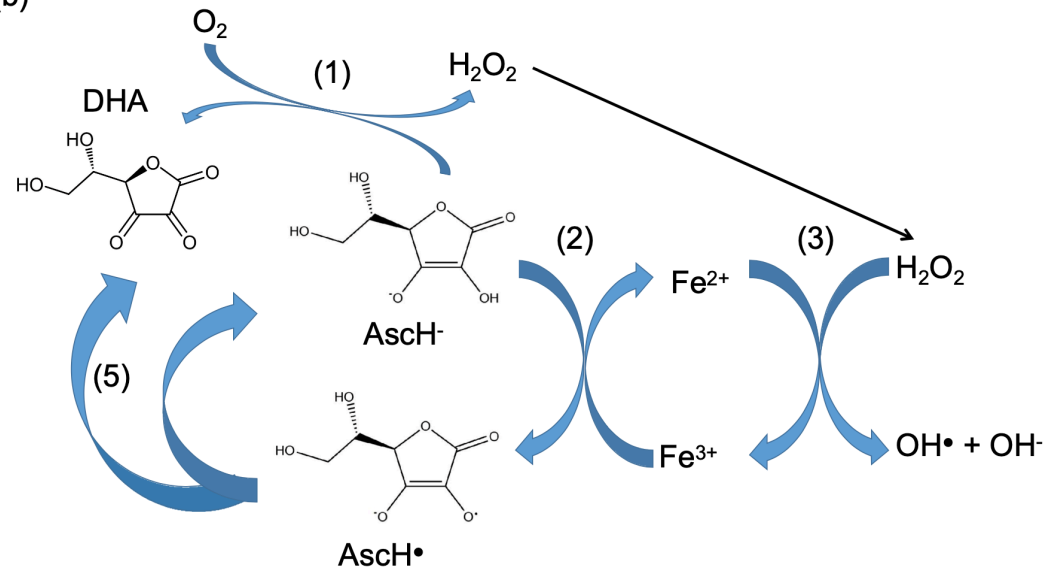


**Figure 2.** The generation of ROS by ascorbate presented in (a) steps of reactions and (b) schematic diagram. Ascorbate ($AscH^-$) could act as a reducing agent to generate hydrogen peroxide ($H_2O_2$) by reducing singlet oxygen (reaction 1). $AscH^-$ could also accelerate the Fenton reaction by reducing ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$), generating hydroxyl radicals ($OH^\bullet$) and ascorbyl radicals ($AscH^\bullet$) (reactions 2 and 3). Two $AscH^\bullet$ molecules could undergo a disproportionation reaction to form $AscH^-$ and dehydroascorbate (reaction 5).

On the other hand, ascorbate acts as a cofactor for different types of α-ketoglutarate-dependent dioxygenase (αKGDD)[21]. The αKGDDs depend on 2-oxoglutarate as a co-substrate and directly bind to $Fe^{2+}$ to be active. Ascorbate likely acts as a reducing agent to reduce the $Fe^{3+}$ to $Fe^{2+}$, which is required for activation of the αKGDDs[19]. Ascorbate is oxidized to ascorbyl radicals in the reaction[22]. Several important αKGDDs include the ten-eleven translocation methylcytosine dioxygenase (TET) enzymes which are involved in DNA demethylation, the HIF hydroxylases which are involved in the ubiquitination and proteasomal degradation of HIF-1α, and the jumonji C domain-containing histone demethylases (JHDMs) which are important in histone demethylation[13].

## 2. Mechanisms of action of ascorbate

Ascorbate potentially fights against cancers via multiple mechanisms (Figure 3). Ascorbate shows various favorable features in anti-neoplastic treatment as a selectively cytotoxic agent and a targeted therapy. There are also immunomodulatory effects mediated by ascorbate which are potentially important in immunity against cancers. Although there are different potential mechanisms of action of ascorbate, the different mechanisms likely coexist *in vivo* and thus ascorbate should be considered as a multitargeting agent.

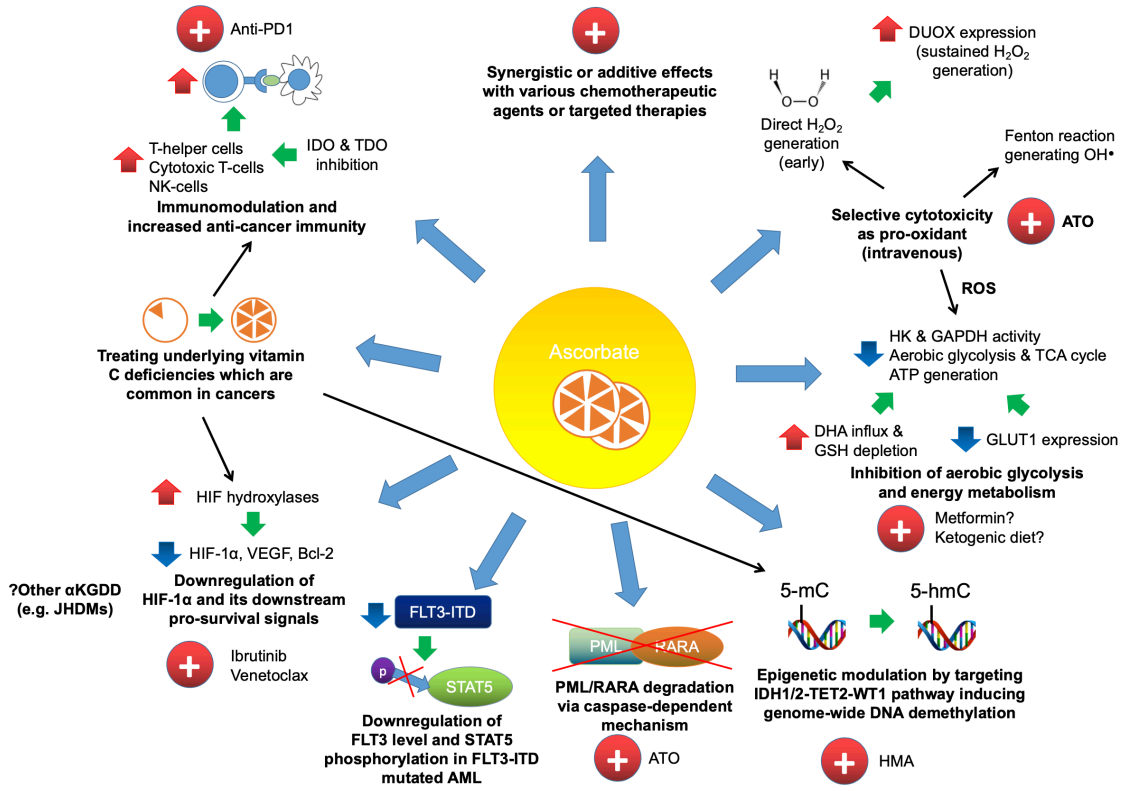


**Figure 3.** The major mechanisms of action of ascorbate against cancers. Ascorbate acts as a multitargeting agent that produces its anti-cancer effects via multiple mechanisms including (i) selective cytotoxicity via reactive oxygen species generation due to its pro-oxidant properties at high concentration; (ii) inhibition of aerobic glycolysis (via GAPDH) and TCA cycle with consequent reduced ATP generation; (iii) epigenetic modulation by targeting IDH1/2-TET2-WT1 pathway and inducing DNA demethylation; (iv) caspase-dependent degradation of PML/RARA oncoprotein in acute promyelocytic leukemia (APL); (v) downregulation of FLT3 protein and phosphorylation of STAT5 in FLT3-ITD mutated acute myeloid leukemia (AML); (vi) downregulation of HIF-1a and its downstream pro-survival signals; (vii) treating underlying vitamin C deficiencies common in hematological malignancies; (viii) immunomodulation and increased anti-cancer immunity; (ix) synergistic or additive effects with various chemotherapeutic agents or targeted therapies (with the likely major mechanism of synergy in different combinations illustrated). Different mechanisms likely occur simultaneously, although the likely mechanism of synergy in different combinations may differ.

### *2.1. Selective cytotoxicity of ascorbate as pro-oxidant and inhibition of Warburg effect*

The mechanisms of pro-oxidant activity of ascorbate at high concentrations are complex. The first mechanism depends on its ability as a reducing agent, which generates $H_2O_2$ from singlet oxygen and accelerates the Fenton reaction with consequent generation of hydroxyl radicals leading to a paradoxical


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pro-oxidant effect (Figure 2)[18][19]. Tumor cells also contain a higher level of labile iron than normal cells, favoring higher ROS generation[23]. The direct addition of $H_2O_2$ also produced cytotoxicity on lymphoma cells similar to that seen after the addition of a high concentration of $AscH^-$, suggesting the role of $H_2O_2$ in high-concentration ascorbate-induced cytotoxicity[24]. Since $H_2O_2$ is membrane-permeant, $H_2O_2$ could potentially target cancer cells both extracellularly such as membrane lipid, or intracellularly such as DNA, DNA repair proteins, and mitochondria[24]. The second mechanism depends on the $H_2O_2$-dependent activation of the plasma membrane-associated dual oxidases (DUOX) 1 and 2, members of the NADPH oxidase (NOX) family of enzymes[25]. DUOX1 and 2 contain a peroxidase-like domain that could produce $H_2O_2$, making them unique in the NOX family of enzymes[26]. $H_2O_2$ directly generated by high-concentration $AscH^-$ could enhance DUOX expression in the tumor cells, which could sustain the generation of $H_2O_2$ and increase oxidative stress even if the $AscH^-$ was removed after exposure for 1 hour[25]. Both mechanisms could contribute to the pro-oxidant cytotoxic effect of ascorbate, with a direct generation of ROS by ascorbate as the short-term effect and the $H_2O_2$-dependent DUOX activation and further $H_2O_2$ generation as the long-term effect. From the study by Kawada et al. using different leukemia cell lines, the cytotoxic effects of ascorbate were observed when ascorbate concentration was ≥280 μM[27]. The direct addition of DHA could not produce the cytotoxic effects as expected for ascorbate at the same concentrations, suggesting that extracellular ascorbate is predominating in producing the cytotoxic effects rather than DHA or intracellular ascorbate[24].

The selective toxicity of ascorbate probably depends on differential modulation of cellular responses to ROS in normal and cancer cells[28]. The toxicity of ascorbate was observed in acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL) cell lines but not in normal CD34+ HSCs *in vitro*[29]. Mastrangelo et al. showed that different human leukemia cell lines, including APL cell lines which are resistant to all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO), are sensitive to a high concentration of ascorbate (average 50% lethal concentration of 3 mM)[30]. Normal cells and cord blood CD34+ HSCs have catalase activity of about four times higher than tumor cells and different leukemia cell lines[27][31]. Catalase is essential for $H_2O_2$ removal, especially when $H_2O_2$ concentration is >10 μM[31]. The addition of catalase abrogates the high-concentration (≥280 μM) ascorbate-induced apoptosis of the leukemia cell lines[27]. These study results provided insight into why the normal cells are spared from cytotoxicity *in vitro*.

### *2.2. Metabolic alteration and inhibition of Warburg effect*

Ascorbate also potentially alters the metabolism of cancer via intracellular mechanisms. DHA is reduced back to ascorbate after entering cancer cells via GLUT1 at the expense of intracellular GSH and NADPH[17]. The increased intracellular ROS due to high-concentration ascorbate could also lead to DNA damage and activation of the DNA repair protein poly ADP ribose polymerase (PARP) which consumes nicotinamide adenine dinucleotide ($NAD^+$)[17]. These would lead to GSH, NADPH, and $NAD^+$ depletion[17][32]. Leukemic cells showed dependence on aerobic glycolysis (also known as the Warburg effect) similar to other cancer cells[33].Treatment with ascorbate was shown to inactivate a glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), via ROS-dependent S-glutathionylation and $NAD^+$ depletion[17][32]. Ascorbate was also shown to inhibit hexokinase (HK) in the glycolytic pathway, likely via an irreversible inhibition of HK by DHA[34][35]. On the other hand, Banella et al. found that 1mM ascorbate significantly decreased GLUT1 expression levels in AML cell lines, further reducing glucose uptake by the leukemic cells[34]. The reduced glucose uptake, the accumulation of upstream metabolites in the glycolysis pathway and tricarboxylic acid (TCA) cycle, and the consequent decreased adenosine triphosphate (ATP) level could contribute to cancer cell death[32][34]. The leukemic cells are shown to be more sensitive to the blockade of aerobic glycolysis than normal HSCs, suggesting a potential selective cytotoxicity by inhibiting cancer cell energy metabolism[33].

### *2.3. Ascorbate as an epigenetic regulator and a potential targeted therapy*

Aberrant DNA methylation and epigenetic alterations are recognized pathogenic mechanisms in hematological malignancies[36][37]. Genome-wide and targeted analyses from next-generation sequencing have identified mutations important in inducing DNA hypermethylation[38]. In myeloid neoplasms, mutations involving TET2, isocitrate dehydrogenases 1 and 2 (IDH1/2), and Wilms tumor 1 (WT1) are implicated in the loss of function of the TET2 enzyme resulting in widespread gene promoter hypermethylation (Figure 4)[36][39][40]. On the other hand, TET2 mutations are also common in lymphomas such as angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), especially those expressing follicular T-helper cell markers[41]. Ascorbate is an epigenetic modulator promoting active genome-wide DNA demethylation through its effects on TET enzymes, a family of αKGDD (Figure 4)[42]. Ascorbate consistently causes DNA demethylation of 1847 genes in human embryonic stem cells[43]. Ascorbate could increase the dioxygenase activity of TET2 by reducing

the $Fe^{3+}$ to $Fe^{2+}$, which binds to the catalytic site of TET2 and activates TET2[44]. Therefore, ascorbate could potentially relieve some biological consequences of loss of function of TET2.

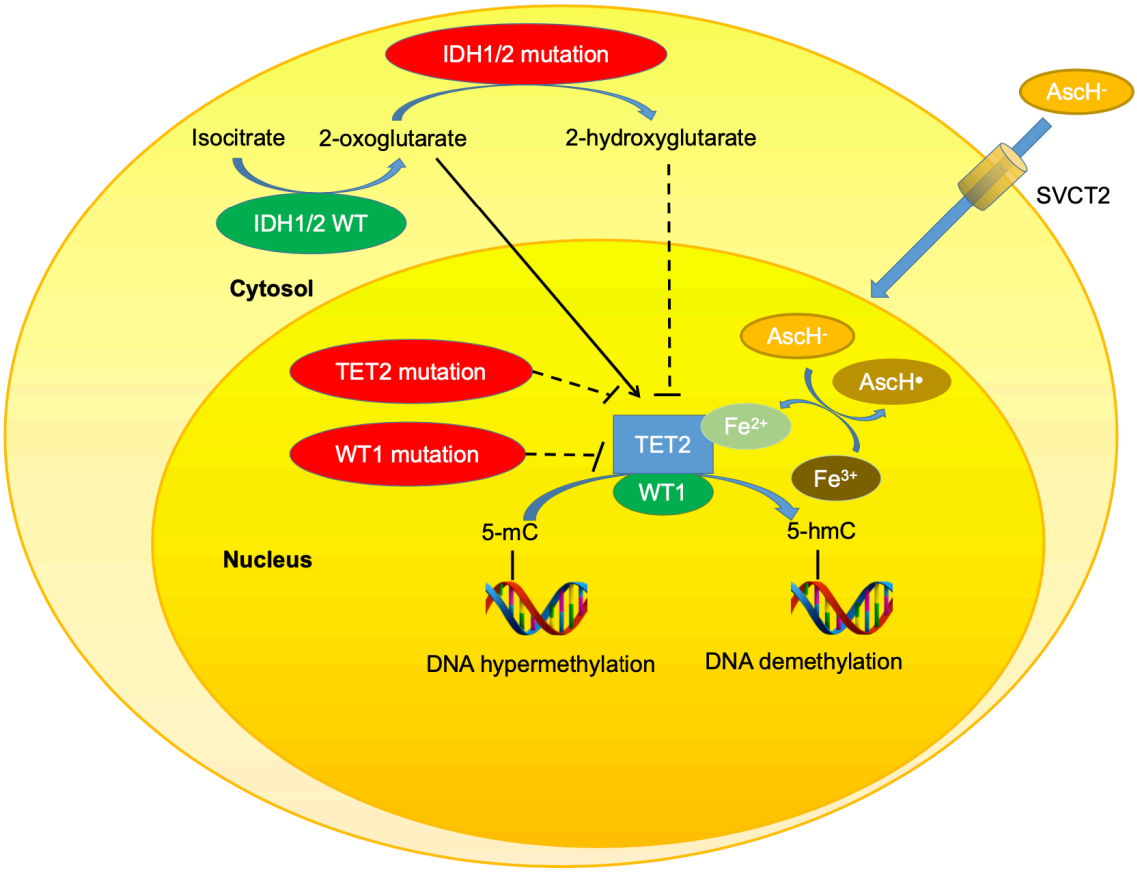


**Figure 4.** The mechanism of ascorbate ($AscH^-$) in targeting the mutations in the IDH1/2-TET2-WT1 pathway. Gain-of-function mutations in IDH1 or IDH2 lead to the abnormal generation of 2-hydroxyglutarate which inhibits the TET2 enzyme activity. Loss-of-function mutations in TET2 lead to decreased TET2 activity and impaired DNA demethylation. Loss-of-function mutations in WT1 impair the recruitment of TET2 to WT1-targeted genes which causes hypermethylation of the genes and reduced expression. Ascorbate likely acts as a reducing agent by reducing ferric iron ($Fe^{3+}$) to the ferrous state ($Fe^{2+}$), which can then directly bind to and activate TET2.

Apart from the effect on the IDH1/2-TET2-WT1 pathway, ascorbate could also act on other potential targets. Ascorbate was found to induce caspase-dependent degradation of promyelocytic leukemia/retinoic acid receptor alpha (PML/RARA) fusion protein, the disease-specific target in APL[29]. Therefore, ascorbate could be also considered a targeted therapy for APL which would be discussed in the later section (section 5,1). On the other hand, 3-5 mM ascorbate was found to downregulate Fms-like tyrosine kinase 3 (FLT3)

protein level and STAT5a/b phosphorylation in AML cell line with FLT3 internal tandem duplication (FLT3-ITD) mutation[29]. The potential of ascorbate as a FLT3 inhibitor is worth further exploration.

### *2.4. Ascorbate regulation of HIF hydroxylases and other αKGDD enzymes*

Ascorbate is also a cofactor of HIF hydroxylases (another class of αKGDD enzymes)[45]. HIF hydroxylases induce recognition of HIF-1α by the von Hippel-Lindau (VHL) protein, leading to its ubiquitination and proteasomal degradation. HIF-1α is often expressed in tumor cells and different leukemia cell lines[13][27]. Achieving a high concentration of ascorbate, or even restoring a physiological level of ascorbate by oral ascorbate supplementation, could potentially downregulate HIF-1α and its downstream pro-survival proteins such as vascular endothelial growth factor (VEGF) and the Bcl-2 family of anti-apoptotic proteins as shown in both *in vitro* and *in vivo* studies[27][45][46].

The JHDMs are another class of αKGDD important in histone demethylation and regulation of gene expression[47]. The JHDMs are characterized by the presence of the Jumonji C domain, a demethylase signature motif that exists in organisms from bacteria to humans[48]. The majority of the JHDMs were found to demethylate specific lysines or arginines in the H3 tail of histone[49]. KDM3B and KDM6A are tumor suppressor JHDM genes that are potentially important in AML[50]. 13% of AML showed decreased KDM3B expression and 1% of AML showed heterozygous loss-of-function KDM6A mutations[50]. However, KDM3B upregulation was also found to induce lmo2 oncogene expression in acute lymphoblastic leukemia[51]. On the other hand, some of the JHDMs such as KDM2B were found to be highly expressed in AML and involved in leukemogenesis[50][52]. Therefore, the role of JHDMs is likely complex and context-dependent in hematological malignancies and requires further investigations.

### *2.5. Ascorbate deficiencies in hematological malignancies*

Multiple studies have also shown that ascorbate depletion (<23 μM) is common in patients with hematological malignancies[53][54][55]. The ascorbate deficiency could further exacerbate the loss of function of TET2. On the other hand, supplementation with oral ascorbate was sufficient to prevent leukemogenesis in $Gulo^{-/-}$ mice transplanted with $TET2^{\Delta/+}$;$FLT3^{ITD/+}$ leukemic cells[15]. Restoring the physiological level of ascorbate by oral ascorbate supplementation can also downregulate HIF-1α in a mouse model[46]. Since the physiological levels of ascorbate could be achievable by oral ascorbate supplementation, it could be a simple way to potentially improve patient outcomes in hematological malignancies. Further studies are required to determine in what circumstances attaining ascorbate level above physiological range by IV ascorbate is preferred over physiological ascorbate level.

### *2.6. Immunomodulatory effects of ascorbate*

Most immune cells contain high intracellular ascorbate levels in the millimolar range to maintain the proper functioning of the immune responses[56]. Depletion of ascorbate could lead to lower ascorbate levels inside immune cells[57]. Ascorbate has potentially significant roles in immunity against cancer via multiple pathways involving T-helper 1 cells, cytotoxic T-cells, and natural killer cells[56]. Of note, ascorbate could produce an inhibitory effect on both tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which are involved in the first and rate-limiting step of metabolism from tryptophan to kynurenine[58][59]. TDO and IDO are shown to have a critical role in T-cell immunosuppression and escape of cancer from anti-cancer immunity via tryptophan depletion[58][59][60]. Ascorbate could potentially enhance anti-cancer immunity by inhibiting TDO and IDO.

## 3. Potential mechanisms of resistance to ascorbate therapy

Several preclinical studies described potential mechanisms of resistance to ascorbate therapy. Liu et al. found that several AML and diffuse large B-cell lymphoma (DLBCL) cell lines have reduced SLC2A3 expression leading to fewer GLUT3 receptors, impeded restoration of TET2 activity, and poor response to ascorbate treatment[16]. The same happens in the KG-1 AML cell line with the knockdown of SLC2A3. HIF-1α overexpression or the addition of catalase could also abrogate the effect of high-dose ascorbate *in vitro*[27]. Some AML and APL cell lines show higher basal catalase activity, which could render potential resistance to the pro-oxidant effect of high-dose ascorbate[61]. While the tumor cells may have a single or a few mechanisms of resistance to ascorbate, they would be less likely to evade all the potential mechanisms of action of ascorbate as a multitargeting agent.

## 4. Safety, adverse effects, and precautions for ascorbate therapy

High-dose IV ascorbate and/or oral ascorbate supplementation are widely used by complementary and alternative medicine practitioners. Both IV ascorbate and oral ascorbate are usually reported as safe with no or minimal side effects in general[62][63]. Yet, several reported adverse effects of ascorbate require attention.

### *4.1. Glucose-6-phosphate dehydrogenase (G6PD) deficiency*

There are case reports of methemoglobinemia and hemolytic anemia in patients with G6PD deficiency induced by high-dose IV ascorbate infusion with a dose of 30 g or more[64][65]. In contrast, low- to

intermediate-dose IV ascorbate (up to 2 g daily to 4 times per day) did not cause oxidative hemolysis in patients with G6PD deficiency in case reports[66][67]. To be safe, G6PD screening should be done before high-dose IV ascorbate treatment to avoid harm due to oxidative hemolysis.

### *4.2. Calcium oxalate calculus and oxalate nephropathy (ON)*

Ascorbate could be metabolized to oxalate in the body (Figure 1), thus increasing urine oxalate excretion and the risk of calcium oxalate calculi formation[68]. For uncertain reasons, oral ascorbate supplementation is associated with a mildly increased risk of renal stone formation in men but not women[69]. However, Prier et al. reported no new-onset renal stone formation in a prospective case series with 157 patients (8% of which have a history of renal stones) receiving IV ascorbate for 12 months[70].

On the other hand, oxalate nephropathy is another risk during high or frequent doses of IV ascorbate administration that could lead to acute kidney injury (AKI) and diffuse tubular deposition of calcium oxalate crystals[71][72]. An observational study showed that an IV ascorbate use of 1.5 g or more increases the risk of AKI and in-hospital mortality in patients with sepsis[73]. There are also different case reports of continuous high-dose oral ascorbate supplementation or chronic ingestion of vitamin C-rich food in a large amount causing ON[74][75][76]. Of note, even chronic ingestion of 480-960 mg daily of vitamin C from diets for longer than 3 months could result in ON[75]. Other risk factors for ON include primary hyperoxaluria, fat malabsorption, gastrointestinal diseases or surgery, ethylene glycol ingestion, ingestion of vitamin C-rich or oxalate-rich food, chronic vitamin C supplementation, and pre-existing chronic kidney diseases[72].

For prudence, it is best to avoid giving ascorbate to patients with a history of calcium oxalate calculi, ON, or chronic kidney disease. A high fluid intake, a low oxalate diet, and oral prophylaxis with magnesium could reduce urinary oxalate levels and the risk of calcium oxalate calculi formation or ON[72][77]. Avoid unbalanced diets that are rich in oxalate or vitamin C. Although vitamin B6 may also reduce urinary oxalate levels, a recent mouse model study showed that the leukemic cells in AML may depend on vitamin B6 for proliferation, suggesting against such supplementation[77][78].

### *4.3. Interference on glucometers leading to pseudohyperglycemia*

IV ascorbate could interfere with many point-of-care glucose meters leading to pseudohyperglycemia and causing detrimental effects due to possible inappropriate insulin therapy[79][80]. Patients are clinically well without hyperglycemic symptoms despite a high glucose reading. Clinicians should be aware of the

phenomenon and be cautious about the glycemic control of patients receiving IV ascorbate. Glucometers that are resistant to interference by IV ascorbate are preferred[81].

### *4.4. Antioxidants use*

Ascorbate at millimolar concentrations is known to produce pro-oxidant effects. However, antioxidants such as N-acetylcysteine and GSH could abrogate the pro-oxidant effects of ascorbate *in vitro*[29][32]. Although *in vivo* results are insufficient, the use of antioxidants should be avoided in general to prevent the potential loss of pro-oxidant cytotoxicity of IV ascorbate.

### *4.5. Hemochromatosis and iron chelators*

Ascorbate is well known for its ability to enhance iron absorption[1]. This would be a concern for patients with hemochromatosis since this can exacerbate the iron overload with the possibility of increasing the generation of ROS and tissue damage even with oral ascorbate supplementation[82]. On the other hand, patients with iron overload may be on iron chelators to relieve the iron overload. It could be problematic for patients on IV ascorbate therapy since iron chelators can inhibit $H_2O_2$ generation and abrogate the pro-oxidant anti-cancer activity of ascorbate[19][83] It is generally recommended that ascorbate should be avoided in patients with hemochromatosis and patients on iron chelation.

### *4.6. Precautions with drug administration of IV ascorbate*

There are a few precautions concerning the administration of IV ascorbate. Riordan et al. suggested that IV ascorbate should be mixed with Ringer's lactate and/or sterile water when infused in a larger amount, at a rate not exceeding 1 g of ascorbate per minute since the solution is hypertonic[77]. Minerals such as calcium chloride, magnesium chloride, and potassium chloride have to be added since IV ascorbate could cause a chloride shift which could cause hypochloremia[84]. The dose of IV ascorbate infusion should be gradually increased based on the tolerance of IV ascorbate. Oral ascorbate supplementation could be given when off infusion to avoid scorbutic rebound effects[77]. Discussion with pharmacists and literature review before starting the use of IVAA could maximize the safety of infusions.

## 5. Interaction of ascorbate with chemotherapy, radiotherapy, or targeted therapies

There were multiple preclinical *in vitro* and *in vivo* studies showing synergistic effects or enhanced efficacy of chemotherapy, radiotherapy, or targeted therapies with high-dose ascorbate. The ability of

ascorbate to enhance the efficacy of a multitude of agents is likely related to its multitargeting properties. However, studies on the same drug combinations may yield inconsistent results[85]. Moreover, the effects of different combinations may be specific to the type of tumors tested. We reviewed different combinations of ascorbate and therapies commonly used in hematological malignancies based on the preclinical or clinical studies (Table 1).

### *5.1. Additive effect with ATO in APL*

The discovery and the use of ATO in APL treatment was a major advance in APL treatment. In the AML17 study, treatment of APL with ATO plus ATRA was demonstrated to induce significantly higher 4-year event-free survival (EFS) rate (91% versus 70%, p = 0.002) and 4-year morphological recurrence-free survival (97% versus 78%, p = 0.004) rate compared with ATRA plus idarubicin, with lower 4-year cumulative incidence of morphological relapse (18% versus 1%, p = 0.0007) and molecular relapse (27% versus 0%, p < 0.0001)[86]. Meta-analysis has also shown that ATO plus ATRA with or without chemotherapy was superior to ATRA plus chemotherapy in terms of EFS, overall survival (OS), and CR rate, with no significant differences in early mortality[87]. However, the use of ATO plus ATRA is still not able to cure all patients in APL.

*In vitro* studies showed that high-concentration ascorbate is highly effective in killing APL cell lines (50% inhibitory concentration ($IC_{50}$) of 1.3 ± 0.3 mM in NB4), including APL cells resistant to ATRA or ATO[29][30]. There was also enhanced ROS production in AML and APL cell lines when 3 mM ascorbate was added in combination with 1 μM of ATO[29]. The cell-killing effects were greater in the APL and some of the AML cell lines both of which showed lower catalase levels, suggesting the potential role of ROS in producing the cytotoxic effects in these leukemic cells[29]. High-concentration ascorbate only shows a slightly additive effect with ATO, possibly due to the overlapping effect of both ascorbate and ATO on PML/RARA fusion oncoprotein and promyelocytic leukemia (PML) protein degradation[29].

The possible benefits of adding ascorbate into the combination of ATRA and ATO were therefore explored in clinical trials. Treatment of APL with ATRA, oral ATO, and oral ascorbate (as known as Triple-A therapy) was shown to have an excellent outcome, with leukemia-free survival (LFS) and OS rates of 100% at 3 years and 94.1% at 5 years[88]. Interestingly, oral ascorbate of 1 g/day was used instead of IV ascorbate in these patients. There is an improved outcome in APL using Triple-A therapy compared with the ATRA plus ATO group in the AML17 study, although it is not a head-to-head comparison[86][88]. There may be an additive effect to the ATO treatment by oral ascorbate supplementation since the expected plasma ascorbate concentration achievable by the oral ascorbate would not reach the level required for

producing cytotoxic pro-oxidant effects of ascorbate[27]. It is uncertain whether IV ascorbate instead of oral ascorbate could confer further benefits or lower the ATO dose required. It is also difficult to prove superiority given the excellent outcome with the current Triple-A therapy.

### *5.2. Synergy with hypomethylating agents in AML*

Liu et al. found that adding a physiological concentration of ascorbate (57 μM) has a synergistic effect to inhibit leukemic cell growth with a low dose of 5-aza-2′-deoxycytidine (decitabine), a DNA methyltransferase inhibitor (DNMTi), using cancer cell lines including AML cell line (HL60)[20]. There is synergistic inhibition of cancer cell proliferation and increased apoptosis of cancer cells associated with upregulation of endogenous viral-defense genes leading to increased viral mimicry, which is one of the possible cancer-defense mechanisms. Zhao et al. demonstrated that low-dose IV ascorbate (50-80 mg/kg/day for 9 days) together with a combination of decitabine, cytarabine, aclarubicin, and granulocyte colony-stimulating factor (DCAG) have increased CR rate (79.92% versus 44.11%; p = 0.004) and median OS (15.3 months versus 9.3 months, p = 0.039) compared with DCAG regimen alone[89]. There is no significant increase in toxicity associated with the addition of low-dose IV ascorbate. The leukemia cell lines showed a significant increase in TET2 activity upon treatment with 0.3 mM of ascorbate plus 2.5 μM of decitabine compared with 2.5 μM of decitabine alone (p = 0.003, 0.002 for NB4 and HL60 respectively, compared to decitabine treatment)[20]. A recent double-blinded randomized control trial has shown that oral ascorbate of 500 mg daily plus azacitidine treatment caused a significant increase in the plasma ascorbate level (mean increase 34.85 ± 7.94 μM, p = 0.0004) and increased DNA demethylation compared with azacitidine plus placebo in patients with either myelodysplastic syndrome (MDS), AML, or chronic myelomonocytic leukemia (CMML)[53]. The increase in DNA demethylation was indicated by an increase in the global 5-hydroxymethylcytosine (5-hmC) to 5-methylcytosine (5-mC) ratio (0.037% versus -0.029%, p = 0.041). The effect was independent of the baseline ascorbate level. However, global DNA demethylation with an increased 5-hmC/5-mC ratio may not predict a superior response to treatment. Global DNA methylation analysis cannot assess the specific methylation status in the critical genes or regions of the disease. Therefore, the increased 5-hmC/5-mC ratio itself does not imply a reduction in the size of abnormal clones nor a hematological response[90].

### *5.3. Inhibition of proteasome inhibitors*

Ascorbate could inhibit bortezomib (PS-341), a proteasome inhibitor (PI), among different myeloma and non-hematological cancer lines and is independent of its antioxidant activity[91][92]. Perrone et al. showed that ascorbate inhibits boronate PIs (including bortezomib and MG262) by direct binding and does

not inhibit other non-boronate PIs such as NPI0052, lactacystin, or MG132[91]. By inference, ascorbate would likely abrogate the activity of ixazomib, which is also a boronate PI. However, the effect of ascorbate on carfilzomib, an epoxyketone-based PI, remains uncertain. A combination of ascorbate and PIs should be avoided unless a particular PI is proven not inhibited by ascorbate.

### *5.4. Synergy with melphalan*

Melphalan is a DNA alkylator used in myeloma treatment, either in combination therapy for transplant-ineligible elderly patients or used in high-dose as a myeloablative conditioning regimen for autologous stem cell transplantation[93][94]. High-dose ascorbate has selective toxicity against CD138+ myeloma cells *in vitro* and is synergistic with melphalan in killing myeloma cells *in vivo*[95]. Reduced-dose melphalan could be potentially used without losing efficacy when given in combination with IV ascorbate[95].

### *5.5. Synergy with ibrutinib, idelalisib, and venetoclax in chronic lymphocytic leukemia (CLL)*

Darwiche et al. studied the effect of 250 μM of ascorbate versus vehicle in combination with (i) ibrutinib (Bruton kinase inhibitor), (ii) idelalisib (phosphoinositide 3-kinase inhibitor), or (iii) venetoclax (Bcl-2 inhibitor) using primary CLL B-cells from 40 treatment-naïve CLL patients and two CLL cell lines[83]. They found that ascorbate has a synergistic effect with all three targeted therapies. In particular, ascorbate is demonstrated to downregulate HIF-1α and its downstream pro-survival signals including the anti-apoptotic protein Bcl-2, which may explain the synergistic effect with the Bcl-2 inhibitor venetoclax[27]. On the other hand, the combination of ibrutinib and venetoclax is synergistic *in vitro* and showed promising results in the randomized Phase II CAPTIVATE Study[96][97]. Therefore, ascorbate possibly enhances the effect of ibrutinib by virtue of its effects in downregulating anti-apoptotic proteins such as Bcl-2, in a way similar to venetoclax. Adding ascorbate to CLL therapies should be further explored. On the other hand, the same combinations should also be investigated in other hematological malignancies to determine if the synergistic effect is specific to CLL.

### *5.6. Synergy with immunotherapies*

There is increasing interest in ascorbate as a potential adjunct to immunotherapies. Of note, Luchtel et al. showed a synergistic effect between anti-PD1 and high-dose intraperitoneal injection of ascorbate leading to slower tumor growth (3-4 folds lower tumor weight than in the vehicle, anti-PD1, or ascorbate alone) in A20 lymphoma immunodeficient mouse model[98]. There are enhanced tumor immune recognition, increased intratumoral infiltration of CD8+ T-cells and macrophages, increased activation of

cytotoxic T-cells, NK-cells, and interleukin-12 production by antigen-presenting cells[98]. IDO inhibition was shown to be a promising strategy to enhance the effect of anti-PD1 in solid tumors[99]. Ascorbate could also potentially enhance the effect of anti-PD1 via competitive inhibition of IDO, which could inhibit tryptophan depletion and therefore prevent T-cell immunosuppression and escape of cancer from anti-cancer immunity[58][59][60].

Magrì et al. showed that ascorbate enhances anti-CTLA-4 activity in mouse colorectal, breast, melanoma, and pancreatic cancer models[100]. They also observed that the anti-cancer effect was the greatest when high-dose ascorbate was administered to immunocompetent mice but not to nonobese diabetic/severe combined immunodeficiency mice. The findings suggest that ascorbate has an immunomodulatory function that requires an intact immune system to maximize its benefits. On the other hand, ascorbate is also potentially beneficial in the chimeric antigen receptor (CAR) T-cell manufacturing process[101].

## 6. Ascorbate and treatment-associated side effects

Several studies evaluated the safety of ascorbate and the changes in symptoms related to solid tumors or treatment with chemotherapy and/or radiotherapy[84][102][103]. There are reported improvements in quality of life (QoL) and decreased discomfort, fatigue, and pain in cancer patients based on the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (QLQ-C30). However, these studies were not randomized control trials. Further high-quality evidence is required to study the use of ascorbate in reducing tissue damage and toxicities. There are some validated instruments of QoL assessment specific for diseases such as AML to provide a more objective QoL assessment[104].

## 7. The remaining questions to answer and directions for future research

There are still multiple unresolved questions to answer in the use of ascorbate in hematological malignancies. Some important clinical questions are discussed below to guide future research directions.

### *7.1. What are the optimal doses, frequencies, and duration of therapy?*

The optimal cytotoxic effects of ascorbate may vary among different tumor cells due to differences in sensitivity, but the usual ascorbate level required to achieve cytotoxicity on tumor cells is from more than 250 μM to the millimolar range[27][29][30]. High-dose IV ascorbate of up to 3 g/kg in a single infusion is

common among complementary and alternative medicine (CAM) practitioners[105][106]. However, such high-dose infusions may not provide additional benefits but expose the patients to risks such as oxidative hemolysis, renal stones, and potentially life-threatening ON[64][65][71][72][73][74][75][76]. There is evidence that low-dose IV ascorbate (50-80 mg/kg/day) could also produce significant benefits with HMA and chemotherapy[89]. High-dose IV ascorbate infusions may not be always necessary to produce treatment benefits from the current experience in allopathic medicine[88][89].

On the other hand, ascorbate at high plasma concentrations has a short elimination half-life, and the plasma ascorbate would normalize within 4 to 6 hours even after a high intravenous dose[6]. In contrast, in the *in vitro* studies, the cancer cell lines were often exposed to ascorbate for 24 hours or longer[29][83][89][91]. The duration of exposure to a cytotoxic level of ascorbate required to produce clinical benefits is uncertain. The optimal frequency of ascorbate administration would depend on the duration of the effects of ascorbate *in vivo*. Ascorbate was shown to generate sustained oxidative stress *in vitro* via $H_2O_2$-enhanced DUOX expression leading to further $H_2O_2$ generation[25]. On the other hand, Campbell et al. showed that increased tumor ascorbate level could be maintained by daily administration of IV ascorbate *in vivo*, even though plasma ascorbate level is normalized much earlier[45]. Daily administration of IV ascorbate is associated with slower tumor growth and reduced HIF-1α and VEGF levels, but not with alternate-day administration of IV ascorbate. These findings suggest that IV ascorbate should be given at least daily to maintain its anti-tumor effects. It is contrary to the usual CAM practice of IV ascorbate infusions a few times per week[105]. Further studies such as mouse models would be required to determine if more frequent low to intermediate doses of IV ascorbate sustaining the ascorbate concentration in the millimolar range could produce similar or even superior benefits compared with high-dose but less frequent IV ascorbate infusions. However, the potential risk of more frequent IV ascorbate infusions should also be considered, especially the risk of potentially severe renal complications.

There is also a need to explore whether oral ascorbate could be used instead of IV ascorbate in some circumstances. IV ascorbate would likely require administration in wards or day centers, but oral ascorbate is more convenient for patients. Maintaining a physiological range of plasma ascorbate levels (26-84 μM)may also provide additional benefits to patients since this ascorbate level can produce epigenetic changes and downregulate HIF-1α[46][53]. Further investigation on oral ascorbate supplementation is needed, especially for patients with more indolent diseases such as clonal cytopenia of undetermined significance or low-risk MDS, which are usually outpatients.

### *7.2. Should ascorbate be considered a targeted therapy?*

Das et al. reported the successful use of high-dose IV ascorbate as a single agent to achieve complete remission in AML with TET2 and WT1 mutations, suggesting that ascorbate may be particularly effective in AML with mutations in the IDH1/2-TET2-WT1 pathway[105]. Yet, ascorbate is unique as a multitargeting agent that produces different anti-cancer effects simultaneously, making the addition of ascorbate to various treatment regimens possible. Group analysis on the treatment response to ascorbate plus standard treatment of the same disease with or without aberrations in the IDH1/2-TET2-WT1 pathway would be intriguing. Such information could help determine if aberrations in the IDH1/2-TET2-WT1 pathway predict better response with ascorbate-containing therapy.

### *7.3. What combinations of treatment are of potential?*

Ascorbate has synergistic or additive effects with different agents in numerous *in vitro* studies (Table 1). Further clinical trials of combining ascorbate with such agents would be of interest. For instance, adding oral ascorbate to hypomethylating agents increases DNA demethylation in different types of myeloid cancers[53]. Further studies on the outcome of such a combination are required to confirm its theoretical benefits related to DNA demethylation. On the other hand, there is a synergism between ascorbate and venetoclax, idelalisib, or ibrutinib in CLL *in vitro*[83]. Testing the combination of ascorbate with one or more of such targeted therapies in CLL and other diseases could be of value. Also, the combination of HMA and venetoclax is synergistic, well-tolerated, and effective (CR + CR with incomplete hematologic recovery rate of 73%) in elderly AML[107]. Since there is evidence of synergistic effects between ascorbate and both HMA and venetoclax, the combination of the three agents is potential[20][83][89]. These *in vitro* studies showed that the synergistic effects occur with a low concentration of ascorbate, therefore low- to intermediate-dose of IV ascorbate or simply oral ascorbate may also produce benefits. On the other hand, further investigations on the addition of ascorbate to the treatment of lymphomas that featured a high frequency of TET2 mutations (including AITL, PTCL-NOS) would be intriguing[41]. Combination with immunotherapies such as anti-PD1 is also of high potential given the inhibitory effect of ascorbate on TDO and IDO, as well as the synergistic effect demonstrated in the mouse model[59][98].

Apart from the established treatments of hematological malignancies, some emerging strategies could also potentially combine with ascorbate treatment. Ascorbate potentially targets the Warburg effect of leukemic cells by inhibiting aerobic glycolysis and the TCA cycle[17][32]. Combination with metabolic-targeted treatments such as a ketogenic diet or biguanides has been suggested (Figure 3)[34][108]. Metformin was shown to inhibit mitochondrial ATP generation, inhibit cell proliferation of leukemic cells,

and induce apoptosis in leukemic cells with lower basal AKT phosphorylation and glucose consumption[109]. Combination with metabolic-targeted therapies is an evolving field that remains to be investigated.

The aforementioned combinations are only a few examples of the many possibilities. There are already several registered phases 1 or 2 clinical trials ongoing to study the effect of oral ascorbate or IV ascorbate as monotherapy or in combination with chemotherapy and/or targeted therapies in hematological malignancies (Table 2)[110][111][112][113][114]. However, IV infusion protocols of less than once daily were adopted in some of these clinical trials, which can potentially compromise the anti-tumor effects of IV ascorbate based on the findings in the mouse model[45]. The treatment protocols in clinical trials should be designed concerning the pharmacokinetics and pharmacodynamics of ascorbate to be more revealing.

## Conclusion

Ascorbate has multiple potential benefits in the treatment of hematological malignancies through its multitargeting effects such as selective cytotoxicity as pro-oxidant, metabolic alteration and inhibition of cancer energy metabolism, epigenetic regulation via the IDH1/2-TET2-WT1 pathway, targeting PML/RARA in acute promyelocytic leukemia and FLT3-ITD in acute myeloid leukemia, regulating hypoxia-inducible factor hydroxylases and other α-ketoglutarate-dependent dioxygenases, immunomodulatory effects via multiple mechanisms including IDO and TDO inhibition, correction of vitamin C deficiencies common in hematological malignancies, and producing synergistic effects with numerous chemotherapeutic agents and targeted therapies. There are also proven benefits of adding ascorbate in some of the treatments of hematological malignancies. Unlike other novel targeted therapies, ascorbate is much more affordable to patients. Adding ascorbate to different standard and novel targeted therapies should be further investigated.

*Tables*

| Drug combination | Drug concentration | Cancer type(s) | Type of Study | Sample size (hematological malignancies only) | Ascorbate concentration / dose | Effects and outcomes | Reference |
|---|---|---|---|---|---|---|---|
| ATO | 1 μM | AML | *in vitro* | 5 cell lines, 48 primary cells | 3-8 mM for 72 hours | Synergy<br>↑ ROS production and apoptosis | Noguera et al. 2017 |
| | 0.5 μM | APL | | | 0-3 mM for 72 hours | Additive<br>↑ ROS production, PML and PML-RARA degradation, and apoptosis | |
| Decitabine | 300 nM | AML | *in vitro* | 1 cell line | 57 μM added daily | Synergy<br>↑ population-doubling time | Liu et al. 2016 |
| | 2.5 μM | AML<br>APL | *in vitro* | 2 cell lines | 0.3 mM for 24, 48, 72 hours | Synergy<br>↑ TET enzyme activity<br>↑ apoptosis | Zhao et al. 2018 |
| Bortezomib | 5-10 nM | MM | *in vitro* | 6 cell lines | 30-500 mM for 24 hours | Inhibitory<br>↑ tumor growth | Perrone et al. 2009 |
| | 0.1 mg/kg 2x/week for 4 weeks | | *in vivo* | 12 Fox-chase SCID mice | 40 mg/kg oral daily for 26 days | | |


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| Drug combination | Drug concentration | Cancer type(s) | Type of Study | Sample size (hematological malignancies only) | Ascorbate concentration / dose | Effects and outcomes | Reference |
|---|---|---|---|---|---|---|---|
| Melphalan | | MM | *in vivo* | 40 NOD.Cγ-Rag1 mice | 4 mg/kg IP daily, 5x/week for 3 weeks | Synergy<br>↓ tumor burden<br>Prolonged survival | Xia et al. 2017 |
| Ibrutinib | 15 μM | CLL | *in vitro* | 2 cell lines, 6 primary cells | 0.1-2 mM for 24 hours | Synergy with all 3 drugs ↓ cell viability | Darwiche et al. 2020 |
| Idelalisib | 50 μM | | | | | | |
| Venetoclax | 10 nM | | | | | | |
| Anti-PD1 | 200 μg IP once every other day | B-cell lymphoma | *in vivo* | 40 BALB/c immunodeficient mice | 4 g/kg IP daily, 5x/week | Synergy<br>↑ tumor macrophage infiltration<br>↑ IL-12 production<br>↑ cytotoxic T-cell and NK-cell activation | Luchtel et al. 2020 |

**Abbreviations.** AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CLL, chronic lymphocytic leukemia; IL-12, interleukin-12; IP, intraperitoneal; MM, multiple myeloma; mM, millimolar; μM, micromolar; nM, nanomolar; PD1, programmed cell death 1; SCID, severe combined immune-deficient; TET, ten eleven translocation.

**Table 1.** Summary of preclinical studies on ascorbic acid interaction with therapies used in hematology malignancies

| NCT number | Cancer type(s) | Study design | Phase | Type of combination | Ascorbate route and dose | Estimated enrollment | Primary outcome(s) |
|---|---|---|---|---|---|---|---|
| NCT03418038 | Relapsed/refractory lymphoma | Randomized, double-blinded, placebo-controlled | 2 | Arm 1: ascorbate + rituximab + combination chemotherapy<br>Arm 2: Placebo + rituximab + combination chemotherapy<br>Arm 3: ascorbate + combination chemotherapy | High dose IV infusion on days 1, 3, 5, 8, 10, 12, 15, 17, and 19 | 147 | Overall response rate |
| NCT03602235 | Relapsed/refractory multiple myeloma | Open-label, single arm | 1 | High-dose ascorbate + low-dose melphalan | IV infusion, 50g/day, 75g/day and 100g/day (3+3 cohort method) | 9 | Number of treatment-related adverse events |
| NCT03999723 | High-risk MDS | Randomized, quadruple-blinded, placebo-controlled | 2 | Arm 1: ascorbate + azacitidine<br>Arm 2: Placebo + azacitidine | Oral, 1g/day, starting day 1 in the 1st azacitidine cycle and continuing until discontinuation of azacitidine or end of study, whichever occurs earlier | 196 | Event-free survival |
| | CMML (10-29% blasts) without MPD | | | | | | |
| | AML with 20-30% blasts | | | | | | |

| NCT number | Cancer type(s) | Study design | Phase | Type of combination | Ascorbate route and dose | Estimated enrollment | Primary outcome(s) |
|---|---|---|---|---|---|---|---|
| NCT03682029 | CCUS<br>Low risk MDS<br>Low grade CMML (CMML-0 or CMML-1) | Randomized, quadruple-blinded, placebo-controlled | 2 | Arm 1: ascorbate Arm 2: Placebo | Oral, 1g/day, for 12 months | 100 | Median Change from Baseline in VAF at 12 Months |
| NCT04689815 | NPM1-mutated AML with positive MRD | Open-label, single arm | 2 | Oral ATO (Arsenol®) (5-10mg per day, from days 1-7 per cycle) + ascorbate + azacitidine | Oral, 1g/day, days 1 to 7 per cycle | 50 | Rate of NPM1 MRD negativity (by RQ-PCR) |

**Abbreviations.** AML, acute myeloid leukemia; ATO, arsenic trioxide; CCUS, clonal cytopenia or undetermined significance; CMML, chronic myelomonocytic leukemia; IV, intravenous; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder; MRD, measurable residual disease; NCT, National Clinical Trial; NPM1, nucleophosmin 1; RQ-PCR, real-time quantitative polymerase chain reaction; VAF, variant allele frequency

**Table 2.** Ongoing clincal trials using ascorbic acid as treatment in hematological malignancies

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### *Author Contributions*

All authors contributed to the study's conception and design, material preparation, data collection, and analysis. All authors wrote the main manuscript text and Lam WK prepared figures 1-4 and tables 1-2. All authors reviewed the manuscript.

### *Data Availability*

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## References


1. ^a, ^b*Padayatty SJ, Levine M. Vitamin C: the known and the unknown and Goldilocks. Oral Dis. 2016;22(6):463-493. doi: 10.1111/odi.12446.*
2. ^*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.*
3. ^*Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proc Natl Acad Sci U S A. 1976;73(10):3685-3689. doi: 10.1073/pnas.73.10.3685.*
4. ^*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(3):137-141. doi: 10.1056/NEJM198501173120301.*
5. ^*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.*
6. ^a, ^b*Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004;140(7):533-7. doi: 10.7326/0003-4819-140-7-200404060-00010.*
7. ^*MacDonald L, Thumser AE, Sharp P. Decreased expression of the vitamin C transporter SVCT1 by ascorbic acid in a human intestinal epithelial cell line. Br J Nutr. 2002;87(2):97-100. doi: 10.1079/BJN2001492.*
8. ^*Haworth WN, Hirst EL. Synthesis of ascorbic acid. J Soc Chem Ind (Lond) 1933;52:645–647.*
9. ^*Chambial S, Dwivedi S, Shukla KK, John PJ, Sharma P. Vitamin C in disease prevention and cure: an overview. Indian J Clin Biochem. 2013;28(4):314-28. doi: 10.1007/s12291-013-0375-3.*

10. ^a, ^b *Linster CL, Van Schaftingen E. Vitamin C. Biosynthesis, recycling and degradation in mammals. FEBS J. 20 07;274(1):1-22. doi: 10.1111/j.1742-4658.2006.05607.x.*

11. ^ *Yang H. Conserved or lost: molecular evolution of the key gene GULO in vertebrate vitamin C biosynthesis. Bi ochem Genet. 2013;51(5-6):413-425. doi: 10.1007/s10528-013-9574-0.*

12. ^ *Drouin G, Godin JR, Pagé B. The genetics of vitamin C loss in vertebrates. Curr Genomics. 2011;12(5):371-378. doi: 10.2174/138920211796429736.*

13. ^a, ^b, ^c, ^d, ^e *Giansanti M, Karimi T, Faraoni I, Graziani G. High-Dose Vitamin C: Preclinical Evidence for Tailorin g Treatment in Cancer Patients. Cancers (Basel). 2021;13(6):1428. doi: 10.3390/cancers13061428.*

14. ^ *DiLabio GA, Wright JS. Hemiketal formation of dehydroascorbic acid drives ascorbyl radical anion dispropor tionation. Free Radic Biol Med. 2000;29(5):480-5. doi: 10.1016/s0891-5849(00)00357-9.*

15. ^a, ^b *Agathocleous M, Meacham CE, Burgess RJ, Piskounova E, Zhao Z, Crane GM, et al. Ascorbate regulates hae matopoietic stem cell function and leukaemogenesis. Nature. 2017;549(7673):476-481. doi: 10.1038/nature2 3876.*

16. ^a, ^b *Liu J, Hong J, Han H, Park J, Kim D, Park H, et al. Decreased vitamin C uptake mediated by SLC2A3 promot es leukaemia progression and impedes TET2 restoration. Br J Cancer. 2020;122(10):1445-1452. doi: 10.1038/s 41416-020-0788-8.*

17. ^a, ^b, ^c, ^d, ^e, ^f *Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K, et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science. 2015;350(6266):1391-6. doi: 10.1126/scienc e.aaa5004.*

18. ^a, ^b *Kramarenko GG, Hummel SG, Martin SM, Buettner GR. Ascorbate reacts with singlet oxygen to produce hy drogen peroxide. Photochem Photobiol. 2006;82(6):1634-7. doi: 10.1562/2006-01-12-RN-774.*

19. ^a, ^b, ^c, ^d *Timoshnikov VA, Kobzeva TV, Polyakov NE, Kontoghiorghes GJ. Redox Interactions of Vitamin C and I ron: Inhibition of the Pro-Oxidant Activity by Deferiprone. Int J Mol Sci. 2020;21(11):3967. doi: 10.3390/ijms2 1113967.*

20. ^a, ^b, ^c, ^d *Liu M, Ohtani H, Zhou W, Ørskov AD, Charlet J, Zhang YW, et al. Vitamin C increases viral mimicry ind uced by 5-aza-2'-deoxycytidine. Proc Natl Acad Sci U S A. 2016;113(37):10238-10244. doi: 10.1073/pnas.1612 262113.*

21. ^ *Ozer A, Bruick RK. Non-heme dioxygenases: cellular sensors and regulators jelly rolled into one? Nat Chem Biol. 2007;3(3):144-53. doi: 10.1038/nchembio863.*

22. ^ *Du J, Cullen JJ, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochim Biophys Acta. 2012;1826(2):443-57. doi: 10.1016/j.bbcan.2012.06.003.*

23. ^McCarty MF, Contreras F. Increasing Superoxide Production and the Labile Iron Pool in Tumor Cells may Sensitize Them to Extracellular Ascorbate. Front Oncol. 2014;4:249. doi: 10.3389/fonc.2014.00249.

24. a, b, cChen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR, 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 U S A. 2005;102(38):13604-9. doi: 10.1073/pnas.0506390102.

25. a, b, cGibson AR, O'Leary BR, Du J, Sarsour EH, Kalen AL, Wagner BA, et al. Dual Oxidase-Induced Sustained Generation of Hydrogen Peroxide Contributes to Pharmacologic Ascorbate-Induced Cytotoxicity. Cancer Res. 2020;80(7):1401-1413. doi: 10.1158/0008-5472.CAN-19-3094.

26. ^Meitzler JL, Hinde S, Bánfi B, Nauseef WM, Ortiz de Montellano PR. Conserved cysteine residues provide a protein-protein interaction surface in dual oxidase (DUOX) proteins. J Biol Chem. 2013;288(10):7147-57. doi: 10.1074/jbc.M112.414797.

27. a, b, c, d, e, f, g, h, iKawada H, Kaneko M, Sawanobori M, Uno T, Matsuzawa H, Nakamura Y, et al. High concentrations of L-ascorbic acid specifically inhibit the growth of human leukemic cells via downregulation of HIF-1α transcription. PLoS One. 2013;8(4):e62717. doi: 10.1371/journal.pone.0062717.

28. ^Wei X, Xu Y, Xu FF, Chaiswing L, Schnell D, Noel T, et al. RelB Expression Determines the Differential Effects of Ascorbic Acid in Normal and Cancer Cells. Cancer Res. 2017;77(6):1345-1356. doi: 10.1158/0008-5472.CAN-16-0785.

29. a, b, c, d, e, f, g, h, i, jNoguera NI, Pelosi E, Angelini DF, Piredda ML, Guerrera G, Piras E, et al. High-dose ascorbate and arsenic trioxide selectively kill acute myeloid leukemia and acute promyelocytic leukemia blasts in vitro. Oncotarget. 2017;8(20):32550-32565. doi: 10.18632/oncotarget.15925.

30. a, b, cMastrangelo D, Massai L, Lo Coco F, Noguera NI, Borgia L, Fioritoni G, et al. Cytotoxic effects of high concentrations of sodium ascorbate on human myeloid cell lines. Ann Hematol. 2015;94(11):1807-16. doi: 10.1007/s00277-015-2464-2.

31. a, bDoskey CM, Buranasudja V, Wagner BA, Wilkes JG, Du J, Cullen JJ, et al. Tumor cells have decreased ability to metabolize H2O2: Implications for pharmacological ascorbate in cancer therapy. Redox Biol. 2016;10:274-284. doi: 10.1016/j.redox.2016.10.010.

32. a, b, c, d, eUetaki 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.

33. a, bWang YH, Scadden DT. Targeting the Warburg effect for leukemia therapy: Magnitude matters. Mol Cell Oncol. 2015;2(3):e981988. doi: 10.4161/23723556.2014.981988.

34. a, b, c, dBanella C, Catalano G, Travaglini S, Pelosi E, Ottone T, Zaza A, et al. Ascorbate Plus Buformin in AML: A Metabolic Targeted Treatment. Cancers (Basel). 2022;14(10):2565. doi: 10.3390/cancers14102565.

35. ^Fiorani M, De Sanctis R, Scarlatti F, Vallorani L, De Bellis R, Serafini G, et al. Dehydroascorbic acid irreversibl y inhibits hexokinase activity. Mol Cell Biochem. 2000;209(1-2):145-53. doi: 10.1023/a:1007168032289.

36. a, bGill H, Leung AY, Kwong YL. Molecular and Cellular Mechanisms of Myelodysplastic Syndrome: Implicatio ns on Targeted Therapy. Int J Mol Sci. 2016;17(4):440. doi: 10.3390/ijms17040440.

37. ^Shenoy N, Bhagat T, Nieves E, Stenson M, Lawson J, Choudhary GS, et al. Upregulation of TET activity with a scorbic acid induces epigenetic modulation of lymphoma cells. Blood Cancer J. 2017;7(7):e587. doi: 10.1038/bc j.2017.65.

38. ^Zhang L, Padron E, Lancet J. The molecular basis and clinical significance of genetic mutations identified in myelodysplastic syndromes. Leuk Res. 2015;39(1):6-17. doi: 10.1016/j.leukres.2014.10.006.

39. ^Rampal R, Alkalin A, Madzo J, Vasanthakumar A, Pronier E, Patel J, et al. DNA hydroxymethylation profiling reveals that WT1 mutations result in loss of TET2 function in acute myeloid leukemia. Cell Rep. 2014;9(5):184 1-1855. doi: 10.1016/j.celrep.2014.11.004.

40. ^Sinha S, Thomas D, Yu L, Gentles AJ, Jung N, Corces-Zimmerman MR, et al. Mutant WT1 is associated with D NA hypermethylation of PRC2 targets in AML and responds to EZH2 inhibition. Blood. 2015;125(2):316-326. doi: 10.1182/blood-2014-03-566018.

41. a, bLemonnier F, Couronné L, Parrens M, Jaïs JP, Travert M, Lamant L, et al. Recurrent TET2 mutations in peri pheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. Blood. 2012;120 (7):1466-9. doi: 10.1182/blood-2012-02-408542.

42. ^Minor EA, Court BL, Young JI, Wang G. Ascorbate induces ten-eleven translocation (Tet) methylcytosine dio xygenase-mediated generation of 5-hydroxymethylcytosine. J Biol Chem. 2013;288(19):13669-13674. doi: 1 0.1074/jbc.C113.464800.

43. ^Chung TL, Brena RM, Kolle G, Grimmond SM, Berman BP, Laird PW, et al. Vitamin C promotes widespread y et specific DNA demethylation of the epigenome in human embryonic stem cells. Stem Cells. 2010;28(10):184 8-55. doi: 10.1002/stem.493.

44. ^Guan Y, Greenberg EF, Hasipek M, Chen S, Liu X, Kerr CM, et al. Context dependent effects of ascorbic acid tr eatment in TET2 mutant myeloid neoplasia. Commun Biol. 2020;3(1):493. doi: 10.1038/s42003-020-01220- 9.

45. a, b, c, dCampbell EJ, Vissers MCM, Wohlrab C, Hicks KO, Strother RM, Bozonet SM, et al. Pharmacokinetic and anti-cancer properties of high dose ascorbate in solid tumours of ascorbate-dependent mice. Free Radic Biol Med. 2016;99:451-462. doi: 10.1016/j.freeradbiomed.2016.08.027.

46. a, b, cCampbell EJ, Vissers MC, Bozonet S, Dyer A, Robinson BA, Dachs GU. Restoring physiological levels of asc orbate slows tumor growth and moderates HIF-1 pathway activity in Gulo(-/-) mice. Cancer Med. 2015;4(2):

303-314. doi: 10.1002/cam4.349.

47. ^Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. Nat Rev Genet. 2006;7(9):715-27. doi: 10.1038/nrg1945.

48. ^Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature. 2006;439(7078):811-6. doi: 10.1038/nature04433.

49. ^Cloos PA, Christensen J, Agger K, Helin K. Erasing the methyl mark: histone demethylases at the center of cel lular differentiation and disease. Genes Dev. 2008;22(9):1115-40. doi: 10.1101/gad.1652908.

50. a, b, cDas AB, Smith-Díaz CC, Vissers MCM. Emerging epigenetic therapeutics for myeloid leukemia: modulati ng demethylase activity with ascorbate. Haematologica. 2021;106(1):14-25. doi: 10.3324/haematol.2020.259 283.

51. ^Kim JY, Kim KB, Eom GH, Choe N, Kee HJ, Son HJ, et al. KDM3B is the H3K9 demethylase involved in transcri ptional activation of lmo2 in leukemia. Mol Cell Biol. 2012;32(14):2917-33. doi: 10.1128/MCB.00133-12.

52. ^He J, Nguyen AT, Zhang Y. KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation a nd maintenance of acute myeloid leukemia. Blood. 2011;117(14):3869-80. doi: 10.1182/blood-2010-10-3127 36.

53. a, b, c, dGillberg L, Ørskov AD, Nasif A, Ohtani H, Madaj Z, Hansen JW, et al. Oral vitamin C supplementation to patients with myeloid cancer on azacitidine treatment: Normalization of plasma vitamin C induces epigeneti c changes. Clin Epigenetics. 2019;11(1):143. doi: 10.1186/s13148-019-0739-5.

54. ^Huijskens MJ, Wodzig WK, Walczak M, Germeraad WT, Bos GM. Ascorbic acid serum levels are reduced in pat ients with hematological malignancies. Results Immunol. 2016;6:8-10. doi: 10.1016/j.rinim.2016.01.001.

55. ^Sharma A, Tripathi M, Satyam A, Kumar L. Study of antioxidant levels in patients with multiple myeloma. L euk Lymphoma. 2009;50(5):809-815. doi: 10.1080/10428190902802323.

56. a, bAng A, Pullar JM, Currie MJ, Vissers MCM. Vitamin C and immune cell function in inflammation and cance r. Biochem Soc Trans. 2018;46(5):1147-1159. doi: 10.1042/BST20180169.

57. ^Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci U S A. 2001;98(17):9842-9846. doi: 10.1073/pnas.171318198.

58. a, b, cYe Z, Yue L, Shi J, Shao M, Wu T. Role of IDO and TDO in Cancers and Related Diseases and the Therapeu tic Implications. J Cancer. 2019;10(12):2771-2782. doi: 10.7150/jca.31727.

59. a, b, c, dYuasa HJ. Inhibitory effect of ascorbate on tryptophan 2,3-dioxygenase. J Biochem. 2022;171(6):653-6 61. doi: 10.1093/jb/mvac024.

60. a, bChen W. IDO: more than an enzyme. Nat Immunol. 2011;12(9):809-11. doi: 10.1038/ni.2088.

61. ^Coe E, Schimmer AD. Catalase activity and arsenic sensitivity in acute leukemia. Leuk Lymphoma. 2008;49 (10):1976-1981. doi: 10.1080/10428190802353617.

62. ^Hoppe C, Freuding M, Büntzel J, Münstedt K, Hübner J. Clinical efficacy and safety of oral and intravenous vi tamin C use in patients with malignant diseases. J Cancer Res Clin Oncol. 2021;147(10):3025-3042. doi: 10.10 07/s00432-021-03759-4.

63. ^Padayatty SJ, Sun AY, Chen Q, Espey MG, Drisko J, Levine M. Vitamin C: intravenous use by complementary a nd alternative medicine practitioners and adverse effects. PLoS One. 2010;5(7):e11414. doi: 10.1371/journal.po ne.0011414.

64. a, b^Lo YH, Mok KL. High dose vitamin C induced methemoglobinemia and hemolytic anemia in glucose-6-p hosphate dehydrogenase deficiency. Am J Emerg Med. 2020;38(11):2488.e3-2488.e5. doi: 10.1016/j.ajem.202 0.05.099.

65. a, b^Quinn J, Gerber B, Fouche R, Kenyon K, Blom Z, Muthukanagaraj P. Effect of High-Dose Vitamin C Infusio n in a Glucose-6-Phosphate Dehydrogenase-Deficient Patient. Case Rep Med. 2017;2017:5202606. doi: 10.11 55/2017/5202606.

66. ^Rehman A, Shehadeh M, Khirfan D, Jones A. Severe acute haemolytic anaemia associated with severe metha emoglobinaemia in a G6PD-deficient man. BMJ Case Rep. 2018;2018:bcr2017223369. doi: 10.1136/bcr-2017- 223369.

67. ^Rino PB, Scolnik D, Fustiñana A, Mitelpunkt A, Glatstein M. Ascorbic acid for the treatment of methemoglobi nemia: the experience of a large tertiary care pediatric hospital. Am J Ther. 2014 Jul-Aug;21(4):240-3. doi: 1 0.1097/MJT.0000000000000028.

68. ^Baxmann AC, De O G Mendonça C, Heilberg IP. Effect of vitamin C supplements on urinary oxalate and pH in calcium stone-forming patients. Kidney Int. 2003;63(3):1066-1071. doi: 10.1046/j.1523-1755.2003.00815.x.

69. ^Jiang K, Tang K, Liu H, Xu H, Ye Z, Chen Z. Ascorbic Acid Supplements and Kidney Stones Incidence Among Men and Women: A systematic review and meta-analysis. Urol J. 2019;16(2):115-120. doi: 10.22037/uj.v0i0.4 275.

70. ^Prier M, Carr AC, Baillie N. No Reported Renal Stones with Intravenous Vitamin C Administration: A Prospec tive Case Series Study. Antioxidants (Basel). 2018;7(5):68. doi: 10.3390/antiox7050068.

71. a, b^Fontana F, Cazzato S, Giovanella S, Ballestri M, Leonelli M, Mori G, et al. Oxalate Nephropathy Caused by Excessive Vitamin C Administration in 2 Patients With COVID-19. Kidney Int Rep. 2020;5(10):1815-1822. doi: 10.1016/j.ekir.2020.07.008.

72. a, b, c, d^Rosenstock JL, Joab TMJ, DeVita MV, Yang Y, Sharma PD, Bijol V. Oxalate nephropathy: a review. Clin Kidney J. 2021 Aug 12;15(2):194-204. doi: 10.1093/ckj/sfab145.

73. a, b McCune TR, Toepp AJ, Sheehan BE, Sherani MSK, Petr ST, Dodani S. High dose intravenous vitamin C treatment in Sepsis: associations with acute kidney injury and mortality. BMC Nephrol. 2021;22(1):387. doi: 10.1186/s12882-021-02599-1.

74. a, b Gurm H, Sheta MA, Nivera N, Tunkel A. Vitamin C-induced oxalate nephropathy: a case report. J Community Hosp Intern Med Perspect. 2012;2(2). doi: 10.3402/jchimp.v2i2.17718.

75. a, b, c Lamarche J, Nair R, Peguero A, Courville C. Vitamin C-induced oxalate nephropathy. Int J Nephrol. 2011; 2011:146927. doi: 10.4061/2011/146927.

76. a, b Moyses-Neto M, Brito BRS, de Araújo Brito DJ, Barros NDC, Dantas M, Salgado-Filho N, et al. Vitamin C-induced oxalate nephropathy in a renal transplant patient related to excessive ingestion of cashew pseudofruit (Anacardium occidentale L.): a case report. BMC Nephrol. 2018;19(1):265. doi: 10.1186/s12882-018-1060-9.

77. a, b, c, d Riordan HD, Hunninghake RB, Riordan NH, Jackson JJ, Meng X, Taylor P, et al. Intravenous ascorbic acid: protocol for its application and use. P R Health Sci J. 2003;22(3):287-90.

78. ^ Chen CC, Li B, Millman SE, Chen C, Li X, Morris JP 4th, et al. Vitamin B6 Addiction in Acute Myeloid Leukemia. Cancer Cell. 2020;37(1):71-84.e7. doi: 10.1016/j.ccell.2019.12.002.

79. ^ Grzych G, Pekar JD, Chevalier-Curt MJ, Decoin R, Vergriete P, Henry H, et al. Antioxidants other than vitamin C may be detected by glucose meters: Immediate relevance for patients with disorders targeted by antioxidant therapies. Clin Biochem. 2021;92:71-76. doi: 10.1016/j.clinbiochem.2021.03.007.

80. ^ Zhang Y, Huang W. Never a rose without a prick: pseudohyperglycemia when administering high-dose intravenous vitamin C. Crit Care. 2020;24(1):251. doi: 10.1186/s13054-020-02994-4.

81. ^ Vanavanan S, Santanirand P, Chaichanajarernkul U, Chittamma A, Dubois JA, Shirey T, et al. Performance of a new interference-resistant glucose meter. Clin Biochem. 2010;43(1-2):186-192. doi: 10.1016/j.clinbiochem.2009.09.010.

82. ^ Fleming RE, Ponka P. Iron overload in human disease. N Engl J Med. 2012;366(4):348-359. doi: 10.1056/NEJMra1004967.

83. a, b, c, d, e Darwiche W, Gomila C, Ouled-Haddou H, Naudot M, Doualle C, Morel P, 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.

84. a, b 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.

85. ^ 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.*

86. ^a, ^b*Burnett AK, Russell NH, Hills RK, Bowen D, Kell J, Knapper S, et al.; UK National Cancer Research Institute Acute Myeloid Leukaemia Working Group. Arsenic trioxide and all-trans retinoic acid treatment for acute pro myelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. Lancet On col. 2015;16(13):1295-1305. doi: 10.1016/S1470-2045(15)00193-X.*

87. ^*Ma Y, Liu L, Jin J, Lou Y. All-Trans Retinoic Acid plus Arsenic Trioxide versus All-Trans Retinoic Acid plus Ch emotherapy for Newly Diagnosed Acute Promyelocytic Leukemia: A Meta-Analysis. PLoS One. 2016;11(7):e01 58760. doi: 10.1371/journal.pone.0158760.*

88. ^a, ^b, ^c*Gill H, Kumana CR, Yim R, Hwang YY, Chan TSY, Yip SF, et al. Oral arsenic trioxide incorporation into fro ntline treatment with all-trans retinoic acid and chemotherapy in newly diagnosed acute promyelocytic leuk emia: A 5-year prospective study. Cancer. 2019;125(17):3001-3012. doi: 10.1002/cncr.32180.*

89. ^a, ^b, ^c, ^d, ^e*Zhao H, Zhu H, Huang J, Zhu Y, Hong M, Zhu H, et al. The synergy of Vitamin C with decitabine activ ates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid l eukemia. Leuk Res. 2018;66:1-7. doi: 10.1016/j.leukres.2017.12.009.*

90. ^*Voso MT, Santini V, Fabiani E, Fianchi L, Criscuolo M, Falconi G, et al. Why methylation is not a marker pred ictive of response to hypomethylating agents. Haematologica. 2014;99(4):613-619. doi: 10.3324/haematol.20 13.099549.*

91. ^a, ^b, ^c*Perrone G, Hideshima T, Ikeda H, Okawa Y, Calabrese E, Gorgun G, et al. Ascorbic acid inhibits antitumo r activity of bortezomib in vivo. Leukemia. 2009;23(9):1679-86. doi: 10.1038/leu.2009.83.*

92. ^*Zou W, Yue P, Lin N, He M, Zhou Z, Lonial S, et al. Vitamin C inactivates the proteasome inhibitor PS-341 in human cancer cells. Clin Cancer Res. 2006;12(1):273-280. doi: 10.1158/1078-0432.CCR-05-0503.*

93. ^*Auner HW, Iacobelli S, Sbianchi G, Knol-Bout C, Blaise D, Russell NH, et al. Melphalan 140 mg/m2 or 200 m g/m2 for autologous transplantation in myeloma: results from the Collaboration to Collect Autologous Trans plant Outcomes in Lymphoma and Myeloma (CALM) study. A report by the EBMT Chronic Malignancies Wor king Party. Haematologica. 2018 Mar;103(3):514-521. doi: 10.3324/haematol.2017.181339.*

94. ^*Morabito F, Bringhen S, Larocca A, Wijermans P, Victoria Mateos M, Gimsing P, et al. Bortezomib, melphala n, prednisone (VMP) versus melphalan, prednisone, thalidomide (MPT) in elderly newly diagnosed multiple myeloma patients: A retrospective case-matched study. Am J Hematol. 2014 Apr;89(4):355-62. doi: 10.1002/ ajh.23641.*

95. ^a, ^b*Xia J, Xu H, Zhang X, Allamargot C, Coleman KL, Nessler R, et al. Multiple Myeloma Tumor Cells are Select ively Killed by Pharmacologically-dosed Ascorbic Acid. EBioMedicine. 2017 Apr;18:41-49. doi: 10.1016/j.ebio m.2017.02.011.*

96. ^Cervantes-Gomez F, Lamothe B, Woyach JA, Wierda WG, Keating MJ, Balakrishnan K, et al. Pharmacological and Protein Profiling Suggests Venetoclax (ABT-199) as Optimal Partner with Ibrutinib in Chronic Lymphocytic Leukemia. Clin Cancer Res. 2015;21(16):3705-15. doi: 10.1158/1078-0432.CCR-14-2809.

97. ^Wierda WG, Allan JN, Siddiqi T, Kipps TJ, Opat S, Tedeschi A, et al. Ibrutinib Plus Venetoclax for First-Line Treatment of Chronic Lymphocytic Leukemia: Primary Analysis Results From the Minimal Residual Disease Cohort of the Randomized Phase II CAPTIVATE Study. J Clin Oncol. 2021;39(34):3853-3865. doi: 10.1200/JCO.21.00807.

98. a, b, cLuchtel RA, Bhagat T, Pradhan K, Jacobs WR Jr, Levine M, Verma A, et al. High-dose ascorbic acid synergizes with anti-PD1 in a lymphoma mouse model. Proc Natl Acad Sci U S A. 2020;117(3):1666-1677. doi: 10.1073/pnas.1908158117.

99. ^Krähenbühl L, Goldinger SM, Mangana J, Kerl K, Chevolet I, Brochez L, et al. A Longitudinal Analysis of IDO and PDL1 Expression during Immune- or Targeted Therapy in Advanced Melanoma. Neoplasia. 2018;20(2):218-225. doi: 10.1016/j.neo.2017.12.002.

100. ^Magrì A, Germano G, Lorenzato A, Lamba S, Chilà R, Montone M, et al. High-dose vitamin C enhances cancer immunotherapy. Sci Transl Med. 2020;12(532):eaay8707. doi: 10.1126/scitranslmed.aay8707.

101. ^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.

102. ^Takahashi H, Mizuno H, Yanagisawa A. High-dose intravenous vitamin C improves quality of life in cancer patients. Personalized Med Universe. 2012;2(1):49–53.

103. ^Vollbracht C, Schneider B, Leendert V, Weiss G, Auerbach L, Beuth J. Intravenous vitamin C administration improves quality of life in breast cancer patients during chemo-/radiotherapy and aftercare: results of a retrospective, multicentre, epidemiological cohort study in Germany. In Vivo. 2011;25(6):983-990.

104. ^Salas M, Henderson M, Wientzek-Fleischmann A, Islam Z, Tu N, Bilitou A, et al. Validated Instruments of Quality of Life (QOL) in Patients With Acute Myeloid Leukemia (AML) and Other Cancers. Front Pharmacol. 2020;11:1109. doi: 10.3389/fphar.2020.01109.

105. a, b, cDas AB, Kakadia PM, Wojcik D, Pemberton L, Browett PJ, Bohlander SK, 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.

106. ^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.

107. ^DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019;133(1):7-17. doi: 10.1182/blood-2018-08-868752.

108. ^Hunyady J. The Result of Vitamin C Treatment of Patients with Cancer: Conditions Influencing the Effectiveness. Int J Mol Sci. 2022;23(8):4380. doi: 10.3390/ijms23084380.

109. ^Scotland S, Saland E, Skuli N, de Toni F, Boutzen H, Micklow E, et al. Mitochondrial energetic and AKT status mediate metabolic effects and apoptosis of metformin in human leukemic cells. Leukemia. 2013;27(11):2129-38. doi: 10.1038/leu.2013.107.

110. ^ClinicalTrials.gov Identifer: NCT03418038. Ascorbic Acid and Combination Chemotherapy in Treating Patients With Relapsed or Refractory Lymphoma. https://clinicaltrials.gov/ct2/show/NCT03418038

111. ^ClinicalTrials.gov Identifer: NCT03602235. High Dose Ascorbic Acid for Plasma Cell Disorders. https://clinicaltrials.gov/ct2/show/NCT03602235

112. ^ClinicalTrials.gov Identifer: NCT03999723. Combining Active and Passive DNA Hypomethylation (EVI-3). https://clinicaltrials.gov/ct2/show/NCT03999723

113. ^ClinicalTrials.gov Identifer: NCT03682029. Epigenetics, Vitamin C, and Abnormal Blood Cell Formation - Vitamin C in Patients With Low-Risk Myeloid Malignancies (EVITA). https://clinicaltrials.gov/ct2/show/NCT03682029

114. ^ClinicalTrials.gov Identifer: NCT04689815. Oral Arsenic Trioxide for NPM1-mutated AML. https://clinicaltrials.gov/ct2/show/NCT04689815

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