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

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Abstract

Ascorbic acid (AA) therapy in cancer treatment has been highly controversial. Despite the lack of high-quality evidence of its efficacy, complementary or alternative medicine practitioners and physicians have used high-dose intravenous AA (IVAA) therapies for cancer or palliative treatment. AA, which was once out of favor in cancer therapy, is being intensely studied due to more knowledge on the pharmacokinetics properties and anti-cancer effects demonstrated in preclinical studies. On the other hand, there has been more understanding of the pathogenesis of hematological malignancies with next-generation sequencing. There is an increasing number of potential targets for therapies, with AA also being one of the candidates that showed examples of success. The potential of AA therapy in both oral and intravenous formulations in hematological malignancies is reviewed in this article to help identify the current knowledge, the unresolved questions, and future research directions.

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Background

Ascorbic acid (AA), also known as vitamin C, is an essential water-soluble vitamin that possesses antioxidant and enzyme cofactor activity. AA 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 AA as a therapeutic agent for cancer after Pauling and Cameron published the earliest reports of cancer patients benefiting from high-dose intravenous AA (IVAA) about 50

years ago[2][3]. However, the subsequent double-blinded randomized control trial by Mayo clinic showed no effect of high-dose oral AA (OAA) supplementation versus placebo in patients with advanced cancers, which dampened the research interest in AA[4]. Yet, the later pharmacokinetics studies of AA showed a remarkable difference in plasma concentrations of AA between oral versus intravenous administration. Plasma AA concentration cannot exceed 250 μM with high-dose OAA, while plasma AA concentration can reach a level over 15 mM by infusing high-dose IVAA[5][6]. The sodium vitamin C cotransporter (SVCT) 1 expression in intestinal cells would decrease upon exposure to an elevated AA level, limiting the bioavailability of OAA[7]. Together with the results of preclinical studies on the effects of AA on cancer cells, there is a flare of research interest in AA as a potential cancer treatment again. There are also examples of success in using AA, either in oral or intravenous forms, as part of the therapy of hematological malignancies. Yet, a thorough review of AA in hematological malignancies is lacking.

In this article, we review the potential of AA therapy in hematological malignancies based on the current evidence on the mechanisms of action, safety and toxicity profile, and interaction with other treatments. We would discuss the unresolved questions and the future research directions in the field.

1. Mechanisms of action of ascorbic acid

AA fights against cancers via multiple mechanisms (Figure 1). AA shows favorable features in anti-neoplastic treatment as a selectively cytotoxic agent and a targeted therapy. There are also immunomodulatory effects mediated by AA which are potentially important in immunity against cancers.

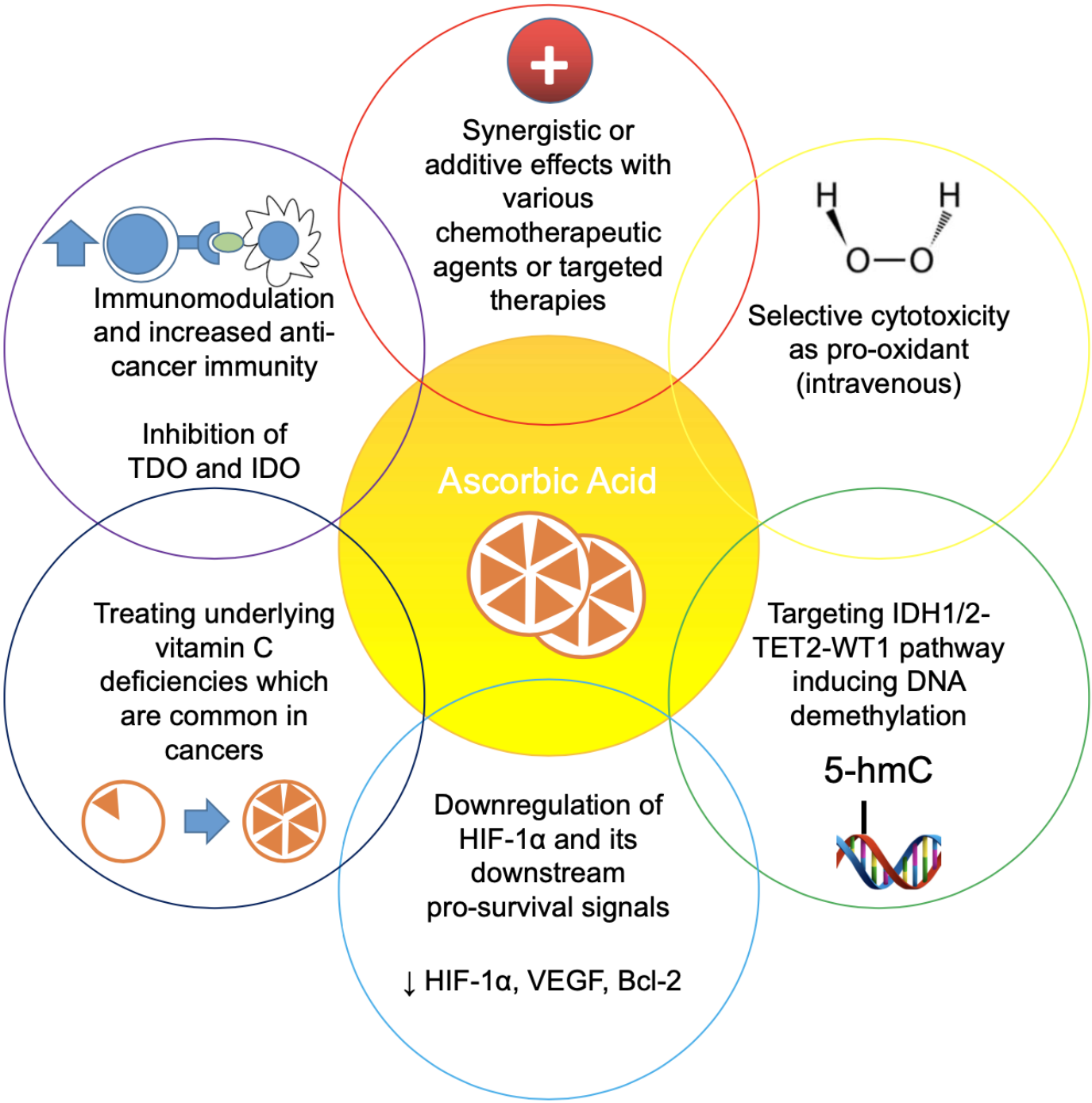

Figure 1. The major mechanisms of action of ascorbic acid against cancers. Ascorbic acid produces its anti-cancer effects via multiple mechanisms including: (i) selective cytotoxicity via reactive oxygen species generation due to its pro-oxidant properties at high concentration; (ii) targeting IDH1/2-TET2-WT1 pathway and induces DNA demethylation; (iii) downregulation of HIF-1a and its downstream pro-survival signals; (iv) treating underlying vitamin C deficiencies which causes impairment of TET2 function; (v) immunomodulation and increased anti-cancer immunity, including TDO and IDO inhibition; (vi) synergistic or additive effects with various chemotherapeutic agents or targeted therapies.

1.1. Selective cytotoxicity of AA as pro-oxidant

AA, at high concentration, acts as a pro-oxidant by its ability to reduce Fe^{3+} to Fe^{2+}, which accelerates the redox cycle of Fe^{3+} and Fe^{2+} in the Fenton reaction, with consequent generation of reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide (H_2O_2)[8][9]. The extracellular ROS can induce cell damage by lipid

peroxidation[9]. AA enters most cells via SVCTs 1 and 2, encoded by SLC23A1 and SLC23A2 genes[10]. Hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) have higher SLC23A2 expression than restricted hematopoietic progenitors. Its oxidized form, dehydroascorbate (DHA), enters cells via glucose transporters (GLUTs), mainly GLUT1 and GLUT3[9][11]. Intracellular DHA is reduced to ascorbate by glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH)[9]. Tumor cells also contain a higher level of labile iron (Fe^{2+}) than normal cells, favoring ROS generation inside the cells[12]. This cytotoxic effect does not occur in the physiological range of AA concentration[13]. The selective toxicity of AA depends on differential modulation of cellular responses to ROS in normal and cancer cells[14]. The selective toxicity of AA occurs in acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL) cell lines but not in normal CD34+ HSCs demonstrated by *in vitro* study[15]. 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 AA (average 50% lethal concentration of 3 mM)[16]. Normal cells and cord blood CD34+ HSCs have catalase activity of about four times higher than tumor cells and different leukemia cell lines[13][17]. Catalase is essential for H_2O_2 removal, especially when H_2O_2 concentration is >10 μM[17]. The addition of catalase abrogates the high-dose (≥280 μM) ascorbate-induced apoptosis of the leukemia cell lines[13]. These *in vitro* study results provided insight into why most patients tolerated high-dose IVAA well.

1.2. AA as a potential targeted therapy

Aberrant DNA methylation and epigenetic alterations are recognized pathogenic mechanisms in hematological malignancies[18][19]. Genome-wide and targeted analyses from next-generation sequencing have identified mutations important in inducing DNA hypermethylation[20]. In myeloid neoplasms, mutations involving ten-eleven translocation methylcytosine dioxygenase (TET) 2, 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 2)[18][21][22]. 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[23]. AA is an epigenetic modulator promoting active genome-wide DNA demethylation through its effects on TET enzymes, a family of α-ketoglutarate-dependent dioxygenases (αKGDD) (Figure 2)[24]. AA consistently causes DNA demethylation of 1847 genes in human embryonic stem cells[25]. AA increases the dioxygenase activity of TET2 by accelerating Fe^{3+} and Fe^{2+} redox cycle, thereby relieving some biological consequences of loss of function of TET2[26].

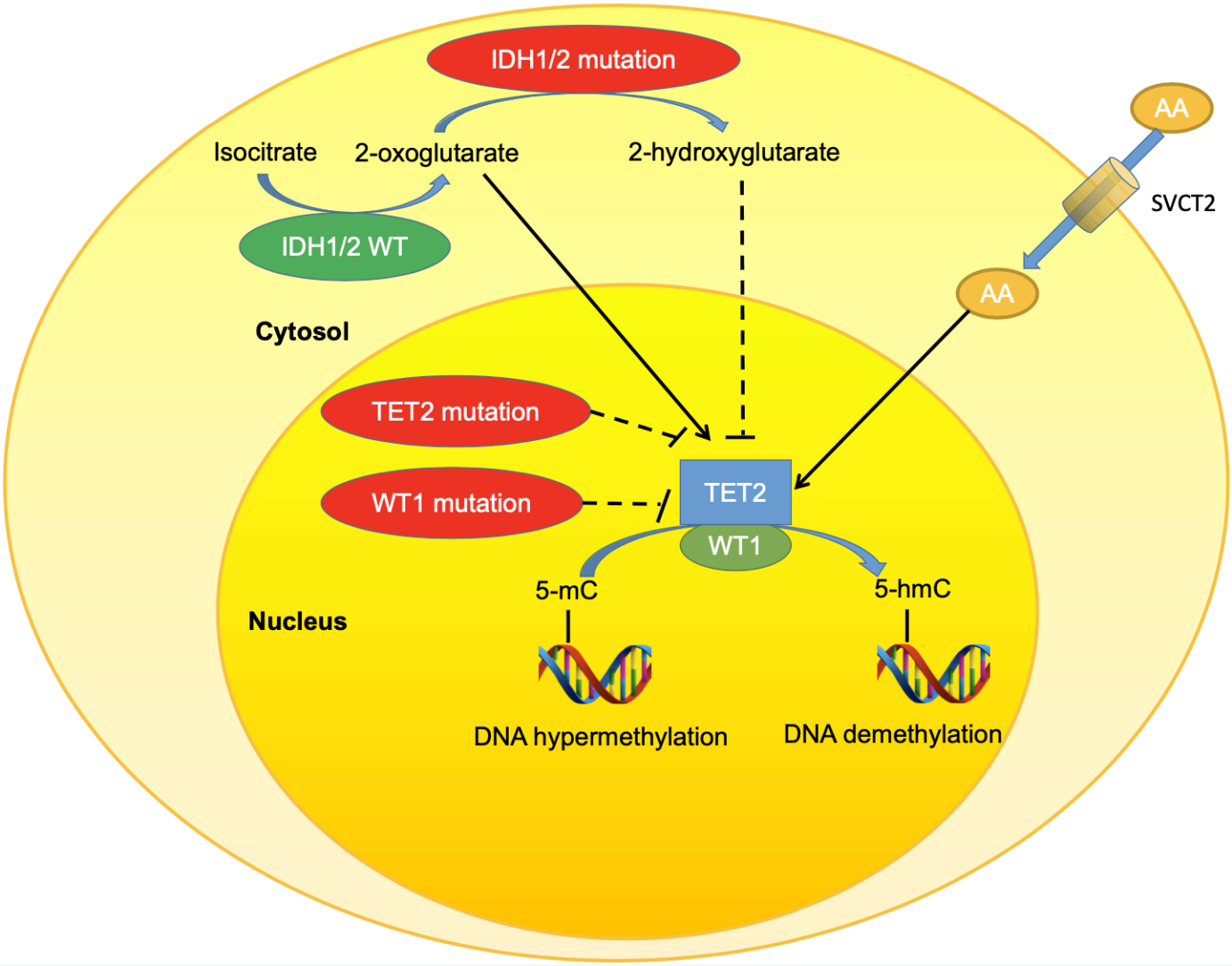

Figure 2. The mechanism of ascorbic acid on targeting the mutations in the IDH1/2-TET2-WT1 pathway. Gain-of-function mutations in IDH1 or IDH2 lead to abnormal generation of 2-hydroxyglutarate which inhibits the TET2 enzyme activity. Loss-of-function mutations in TET2 lead to decreased TET2 activity 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.

1.3. AA regulation of HIF hydroxylases and other αKGDD enzymes

AA is also a cofactor of HIF hydroxylases (another class of αKGDD enzymes)[27]. 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[9][13]. Administration of IVAA, or even restoring a physiological level of AA by OAA supplementation, can 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[13][27][28]. Jumonji C-domain containing histone demethylases (JHDMs) are another class of αKGDD potentially important in histone demethylation[9]. Their role in leukemogenesis requires further investigation.

1.4. AA deficiencies in hematological malignancies

Humans cannot synthesize AA due to the evolutionary loss of function of the enzyme L-gulono-gamma-lactone

oxidase (Gulo), which catalyzes the final step in vertebrate AA biosynthesis[29]. The functional loss of Gulo is common among species with AA rich diet, in which the mutation does not pose a selective disadvantage in general[30]. Multiple studies have also shown that AA depletion (<23 μM) is common in patients with hematological malignancies[31][32][33]. The AA deficiency could further exacerbate the loss of function of TET2. On the other hand, supplementation with OAA was sufficient to prevent leukemogenesis in Gulo$^{-/-}$ mice transplanted with TET2$^{\Delta/+}$;FLT3$^{ITD/+}$ leukemic cells[10]. Restoring the physiological level of AA by OAA supplementation can also downregulate HIF-1α[28]. Since the physiological levels of AA could be achievable by OAA supplementation, it could be a simple way to improve patient outcomes in hematological malignancies by giving OAA supplementation. Further studies are required to determine in what circumstances attaining AA level above physiological range by IVAA would provide additional benefits over physiological AA level.

1.5. Immunomodulatory effects of AA

Most immune cells contain high intracellular AA levels in the millimolar range to maintain the proper functioning of the immune responses[34]. Depletion of AA could lead to lower AA levels inside immune cells[35]. AA has significant roles in immunity against cancer via multiple pathways involving T-helper 1 cells, cytotoxic T-cells, and natural killer cells[34]. Of note, AA produces 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[36][37]. TDO and IDO have a critical role in T-cell immunosuppression and escape of cancer from anti-cancer immunity via tryptophan depletion[36][37][38]. AA is a competitive inhibitor of TDO and IDO, enhancing anti-cancer immunity.

2. Potential mechanisms of resistance to AA therapy

Several preclinical studies described potential mechanisms of resistance to AA 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 and poor response to AA treatment[11]. 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 AA *in vitro*[13]. Some AML and APL cell lines show higher basal catalase activity, which could render potential resistance to the pro-oxidant effect of high-dose AA[39].

3. Safety, adverse effects, and precautions for AA therapy

High-dose IVAA and/or OAA supplementation are widely used by complementary and alternative medicine practitioners. Reviews on the safety of IVAA and OAA showed that AA is well-tolerated with no or minimal side effects in general[40][41]. Yet, several reported adverse effects of AA require attention.

3.1. Glucose-6-phosphate dehydrogenase (G6PD) deficiency

There are case reports of methemoglobinemia and hemolytic anemia in patients with G6PD deficiency induced by high-dose IVAA infusion with a dose of 30 g or more[42][43]. In contrast, low- to intermediate-dose IVAA (up to 2 g daily to

4 times per day) is safe in patients with G6PD deficiency[44][45]. It is coherent with the finding that the pro-oxidant effect of AA predominates in high plasma AA concentration by *in vitro* studies[13]. G6PD screening should be done before high-dose IVAA treatment to avoid harm due to oxidative hemolysis.

3.2. Calcium oxalate calculus and oxalate nephropathy (ON)

AA could be metabolized to oxalate after ingestion, thus increasing urine oxalate excretion and the risk of calcium oxalate calculi formation[46]. OAA supplementation is associated with a mildly increased risk of renal stone formation in men but not women for uncertain reasons[47]. 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 IVAA for 12 months[48]. On the other hand, ON is another risk during high-dose IVAA administration that could lead to acute kidney injury (AKI) and diffuse tubular deposition of calcium oxalate crystals[49][50]. An observational study showed that an IVAA use of 1.5 g or more increases the risk of AKI and in-hospital mortality in patients with sepsis[51].

For prudence, use high-dose AA (either oral or intravenous forms) with caution in patients with a history of calcium oxalate calculi. Avoid giving AA to patients with a history of ON. 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[50][52]. 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[52][53].

3.3. Interference on glucometers leading to pseudohyperglycemia

IVAA can interfere with many point-of-care glucose meters leading to pseudohyperglycemia and causing detrimental effects due to possible inappropriate insulin therapy[54][55]. 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 IVAA. Glucometers that are resistant to interference by IVAA are preferred[56].

3.4. Antioxidants use

AA at millimolar concentrations is known to produce pro-oxidant effects. Antioxidants such as N-acetylcysteine could abrogate the pro-oxidant effects of AA *in vitro*[15]. Antioxidants should be avoided in general to prevent the potential loss of efficacy of IVAA.

3.5. Hemochromatosis and iron chelators

AA 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 OAA supplementation[57]. 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 IVAA therapy since iron chelators can inhibit H_2O_2 generation and abrogate the pro-oxidant anti-cancer activity of AA[8][58]. It is generally recommended that AA should be avoided in patients with hemochromatosis and patients on iron chelation.

3.6. Precautions with drug administration of IVAA

There are a few precautions concerning the administration of IVAA. Riordan et al. suggested that IVAA 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 AA per minute since the solution is hypertonic[52]. Minerals such as calcium chloride, magnesium chloride, and potassium chloride have to be added since IVAA could cause a chloride shift which could cause hypochloremia[59]. The dose of IVAA infusion should be gradually increased based on the tolerance of IVAA. OAA supplementation should be given when off infusion to avoid scorbutic rebound effects[52]. Discussion with pharmacists and literature review before starting the use of IVAA could maximize the safety of infusions.

4. Interaction of AA 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 AA.However, studies on the same drug may yield controversial results[60]. Moreover, the effects of different combinations may be specific to the type of tumors tested. We reviewed different combinations of AA and therapies commonly used in hematological malignancies based on the preclinical or clinical studies (Table 1).

4.1. 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%, $p < 0.0001$)[61]. 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[62]. However, the use of ATO plus ATRA is still not able to cure all patients in APL.

*In vitro*studies showed that high-concentration AA 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[15][16]. High-concentration AA only shows a slightly additive effect with ATO, possibly due to the overlapping effect of both AA and ATO on PML-RARA and PML degradation[15]. The possible benefits of adding AA into the combination of ATRA and ATO were therefore explored. Treatment of APL with ATRA, ATO, and OAA (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[63][64]. Interestingly, OAA of 1 g/day was used instead of IVAA 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[61][63]. There may be an additive effect to the ATO treatment by OAA supplementation since the expected plasma AA concentration achievable by the OAA would not reach the level required for producing cytotoxic pro-oxidant effects of AA[13]. It is

uncertain whether high-dose IVAA instead of OAA could confer further benefits or lower the ATO dose. It is also difficult to prove superiority given the excellent outcome with the current Triple-A therapy.

4.2. Synergy with hypomethylating agents in AML

Liu et al. found that a physiological concentration of AA (26-84 μM) has a synergistic effect with a low dose of 5-aza-2′-deoxycytidine (decitabine), a DNA methyltransferase inhibitor (DNMTi), using cancer cell lines including AML cell line (HL60)[65]. 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 IVAA (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[66]. There is no significant increase in toxicity associated with the addition of low-dose IVAA. The leukemia cell lines showed a significant increase in TET2 activity upon treatment with 0.3 mM of AA 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)[66]. A recent double-blinded randomized control trial has shown that OAA of 500 mg daily plus azacitidine treatment caused a significant increase in the plasma AA 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)[33]. 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 AA 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[67].

4.3. Inhibition of proteasome inhibitors

AA inhibits bortezomib (PS-341), a proteasome inhibitor (PI), among different myeloma and non-hematological cancer lines and independent of its antioxidant activity[68][69]. Perrone et al. showed that AA inhibits boronate PIs (including bortezomib and MG262) by direct binding and does not inhibit other non-boronate PIs such as NPI0052, lactacystin, or MG132[69]. By inference, AA would likely abrogate the activity of ixazomib, which is also a boronate PI. However, the effect of AA on carfilzomib, an epoxyketone-based PI, remains uncertain. A combination of AA and PIs should be avoided unless a particular PI is proven not inhibited by the AA.

4.4. 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[70][71]. High-dose AA has selective toxicity against CD138+ myeloma cells *in vitro* and is synergistic with melphalan in killing myeloma cells *in vivo*[72]. Reduced-dose melphalan could be potentially used without losing efficacy

when given in combination with IVAA[71].

4.5. Synergy with ibrutinib, idelalisib, and venetoclax in chronic lymphocytic leukemia (CLL)

Darwiche et al. studied the effect of 250 μM of AA versus vehicle in combination with (i) ibrutinib (Bruton kinase inhibitor), (ii) idelalisib (phosphoinositide 3-kinase inhibitor), or (iii) venetoclax (BCL2 inhibitor) using primary CLL B-cells from 40 treatment-naïve CLL patients and two CLL cell lines[58]. They found that AA has a synergistic effect with all three targeted therapies. There is also increased cell death when AA is added to fludarabine plus cyclophosphamide compared with vehicle. Adding AA 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.

4.6. Synergy with immunotherapies

There is increasing interest in AA as a potential adjunct to immunotherapies. Of note, Luchtel et al. showed a synergistic effect between anti-PD1 and high-dose intraperitoneal injection of AA leading to slower tumor growth (3-4 folds lower tumor weight than in the vehicle, anti-PD1, or AA alone) in A20 lymphoma immunodeficient mouse model[73]. 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. AA could potentially enhance the effect of anti-PD1 via competitive inhibition of IDO, which is an effective strategy to enhance the effect of anti-PD1 in solid tumors[36][37][38][74].

Magrì et al. showed that AA enhances anti-CTLA-4 activity in mouse colorectal, breast, melanoma, and pancreatic cancer models[75]. They also observed that the anti-cancer effect was the greatest when high-dose AA was administered to immunocompetent mice but not to nonobese diabetic/severe combined immunodeficiency mice. The findings suggest that AA has an immunomodulatory function that requires an intact immune system to maximize its benefits[72]. On the other hand, AA is also potentially beneficial in the chimeric antigen receptor (CAR) T-cell manufacturing process[76].

5. AA and treatment-associated side effects

Several studies evaluated the safety of AA and the changes in symptoms related to solid tumors or treatment with chemotherapy and/or radiotherapy[59][77][78]. There are reported improvements in quality of life and reduced 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 AA in reducing tissue damage and toxicities. There are some validated instruments of quality of life (QoL) assessment specific for diseases such as AML to provide a more objective QoL assessment[79].

6. The remaining questions to answer and directions for future research

There are still multiple unresolved questions to answer in the use of AA in hematological malignancies. Some

important clinical questions are discussed below to guide future research directions.

6.1. What are the optimal doses, frequencies, and duration of therapy?

The optimal cytotoxic effects of AA may vary among different tumor cells due to differences in sensitivity, but the usual AA level required to achieve cytotoxicity on tumor cells is from more than 250 μM to the millimolar range[13][15][16]. High-dose IVAA of up to 3 g/kg in a single infusion is common among CAM practitioners[80][81]. 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[42][43][52]. There is evidence that low-dose IVAA (50-80 mg/kg/day) could also produce significant benefits with HMA and chemotherapy[66]. Such pro-oxidant effects produced by high-dose IVAA infusions may not be always necessary to produce benefits from the current experience in allopathic medicine[63][66].

On the other hand, AA has a short elimination half-life, and the plasma AA would normalize within 4 to 6 hours even after a high intravenous dose[5]. In contrast, in the *in vitro* studies, the cancer cell lines were often exposed to AA for 24 hours or longer[15][58][66][69]. The duration of exposure to a cytotoxic level of AA required to produce clinical benefits is uncertain. The optimal frequency of AA administration would depend on the exposure time to a high AA level necessary for its cytotoxic effects *in vivo*. Campbell et al. showed that increased tumor ascorbate level could be maintained by daily administration of IVAA, even though plasma AA level is normalized much earlier[27]. Daily administration of IVAA is associated with slower tumor growth and reduced HIF-1α and VEGF levels, but not with alternate-day administration of IVAA. These findings suggest that IVAA should be given at least daily to maintain its anti-tumor effects. It is contrary to the usual CAM practice of IVAA infusions a few times per week[80]. Further studies such as mouse models would be required to determine if more frequent low to intermediate doses of IVAA sustaining the AA concentration in the millimolar range could produce similar or even superior benefits compared with high-dose but less frequent IVAA infusions.

There is also a need to explore whether OAA could be used instead of IVAA in some circumstances. IVAA would likely require administration in wards or day centers, but OAA is more convenient for patients. Maintaining a physiological range of plasma AA levels (>23 μM) may also provide additional benefits to patients since this AA level can produce epigenetic changes and downregulate HIF-1α[28][33]. Further investigation on OAA 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.

6.2. Should AA be considered a targeted therapy or an adjunct to chemotherapy?

Das et al. reported the successful use of high-dose IVAA as a single agent to achieve complete remission in AML with TET2 and WT1 mutations, suggesting that AA may be particularly effective in AML with mutations in the IDH1/2-TET2-WT1 pathway[80]. Yet, AA is unique as a multitargeting agent that produces different anti-cancer effects, making the addition of AA to various treatment regimens possible. Group analysis on the treatment response to AA 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 such aberrations predict better outcomes with AA-containing therapy.

6.3. What combinations of treatment are of potential?

AA has synergistic or additive effects with different agents in numerous*in vitro* studies (Table 1). Further clinical trials of combining AA with such agents would be of interest. For instance, adding OAA to hypomethylating agents increases DNA demethylation in different types of myeloid cancers[33]. 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 AA and venetoclax, idelalisib, or ibrutinib in CLL *in vitro*[58]. Testing the combination of AA with 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[82]. Since there is evidence of synergistic effects between AA and both HMA or venetoclax, the combination of the three agents is potential[58][65][66]. These *in vitro* studies showed that the synergistic effects occur with a low concentration of AA, therefore low- to intermediate-dose of IVAA or high-dose OAA may also produce benefits. On the other hand, further investigations on the addition of AA to the treatment of lymphomas that featured a high frequency of TET2 mutations (including AITL, PTCL-NOS) would be intriguing[23]. Combination with immunotherapies such as anti-PD1 is also of high potential given the inhibitory effect of AA on TDO and IDO, as well as the synergistic effect demonstrated in the mouse model[36][73].

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 OAA or IVAA as monotherapy or in combination with chemotherapy and/or targeted therapies in hematological malignancies (Table 2) [83][84][85][86][87]. 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 IVAA based on the findings in the mouse model[27]. The treatment protocols in clinical trials should be designed concerning the pharmacokinetics and pharmacodynamics of AA to be more revealing.

Conclusion

AA has multiple potential benefits in the treatment of hematological malignancies through its multitargeting effects such as selective cytotoxicity with remarkable safety, targeting of IDH1/2-TET2-WT1 pathway and genome-wide DNA demethylation, targeting HIF-1α and other αKGDD enzymes, immunomodulatory effects via multiple mechanisms including TDO and IDO 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 AA in some of the treatments of hematological malignancies. Unlike other novel targeted therapies, AA is much more affordable to patients. Adding AA to different standard and novel targeted therapies should be further investigated.

Tables

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

Drug combination	Drug concentration	Cancer type(s)	Type of Study	Sample size (hematological malignancies only)	AA concentration / dose	Effects and outcomes	Reference
ATO	1 μM	AML	*in vitro*	5 cell lines, 48 primary cells	AA of 3-8 mM for 72 hours	Synergy ↑ apoptosis	Noguera et al.[15]
	0.5 μM	APL			AA of 0-3 mM for 72 hours	Additive ↑ apoptosis	
Decitabine	300 nM	AML	*in vitro*	1 cell line	AA of 57 μM added daily	Synergy ↑ population-doubling time	Liu et al.[65]
	2.5 μM	AML APL	*in vitro*	2 cell lines	AA of 0.3 mM for 24, 48, 72 hours	Synergy ↑ TET enzyme activity ↑ apoptosis	Zhao et al.[66]
Bortezomib	5-10 nM	MM	*in vitro*	6 cell lines	AA of 30-500 mM for 24 hours	Inhibitory ↑ tumor growth	Perrone et al.[69]
	0.1 mg/kg 2x/week for 4 weeks		*in vivo*	12 Fox-chase SCID mice	40 mg/kg oral daily for 26 days		
Melphalan		MM	*in vivo*	40 NOD.Cγ-Rag1 mice	4 mg/kg IP daily, 5x/week for 3 weeks	Synergy ↓ tumor burden Prolonged survival	Xia et al.[72]
Ibrutinib	15 μM	CLL	*in vitro*	2 cell lines, 6 primary cells	AA of 0.1-2 mM for 24 hours	Synergy with all 3 drugs ↓ cell viability	Darwiche et al.[58]
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	AA of 4 g/kg IP daily, 5x/week	Synergy ↑ tumor macrophage infiltration ↑ IL-12 production ↑ cytotoxic T-cell and NK-cell activation	Luchtel et al.[73]

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

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

NCT number	Cancer type(s)	Study design	Phase	Type of combination	AA route and dose	Estimated enrollment	Primary outcome(s)
NCT03418038[83]	Relapsed/refractory lymphoma	Randomized, double-blinded, placebo-controlled	2	Arm 1: AA + rituximab + combination chemotherapy Arm 2: Placebo + rituximab + combination chemotherapy Arm 3: AA + combination chemotherapy	High dose IV infusion on days 1, 3, 5, 8, 10, 12, 15, 17, and 19	147	Overall response rate
NCT03602235[84]	Relapsed/refractory multiple myeloma	Open-label, single arm	1	High-dose AA + low-dose melphalan	IV infusion, 50g/day, 75g/day and 100g/day (3+3 cohort method)	9	Number of treatment-related adverse events
NCT03999723[85]	High-risk MDS CMML (10-29% blasts) without MPD AML with 20-30% blasts	Randomized, quadruple-blinded, placebo-controlled	2	Arm 1: AA + azacitidine Arm 2: Placebo + azacitidine	Oral, 1g/day	196	Event-free survival
NCT03682029[86]	CCUS Low risk MDS Low grade CMML (CMML-0 or CMML-1)	Randomized, quadruple-blinded, placebo-controlled	2	Arm 1: AA Arm 2: Placebo	Oral, 1g/day	100	Median Change from Baseline in VAF at 12 Months
NCT04689815[87]	NPM1-mutated AML with positive MRD	Open-label, single arm	2	Oral ATO (Arsenol®) (5-10mg per day, from days 1-7 per cycle) + AA + azacitidine	Oral, 1g/day	50	Rate of NPM1 MRD negativity (by RQ-PCR)

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

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Author Contributions

All authors contributed to the study's conception and design, material preparation, data collection, and analysis. Lam WK wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

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

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