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

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Abstract

The use of ascorbic acid (AA) in cancer treatment has been a highly controversial area that has led to debates in the medical field. Despite the lack of high quality evidence of its efficacy, high-dose intravenous AA (IVAA) therapies has been used by complementary or alternative medicine practitioners and physicians for cancer or palliative treatment. AA, which was once out of favor in cancer therapy, is now 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 on the pathogenesis of hematological malignancies with the aid of next generation sequencing. There are increasingly number of potential targets for therapies, with AA also being one of the candidates which showed examples of success. The potential of AA therapy including both oral and intravenous AA 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 which possesses antioxidant and enzyme cofactor activity which is involved in many important biological processes including norepinephrine synthesis, collagen hydroxylation, hypoxia-inducible factor (HIF) hydroxylation and regulation as well as promotion of iron absorption[1]. There is a long history of controversy in utilizing AA as a therapeutic agent for cancer since it was first introduced by Pauling and Cameron, who published the earliest reports of cancer patients benefited from high-dose

intravenous AA (IVAA) about 50 years ago[2][3]. However, the interest on AA in cancer therapy was dampened by the results of the double-blind randomized control trial by Mayo clinic which showed no effect of high-dose oral AA (OAA) supplementation versus placebo in patients with advanced cancers.[4] Yet, the later pharmacokinetics studies of AA showed that there is remarkable difference in plasma concentrations of AA between oral versus intravenous administration, in which plasma AA concentration cannot exceed 250 μM with high-dose OAA but plasma AA concentration of >15 mM can be achieved by infusing high-dose IVAA.[5][6] The difference is due to a tight regulatory mechanism of AA uptake by sodium vitamin C cotransporter (SVCT) 1 in small intestine which could be bypassed with IVAA.[7] Together with the results of preclinical studies on the effects of AA on cancer cells, the research interest on AA as potential anti-cancer treatment was ignited again.

In this review, the current evidence on the mechanisms of action, safety and toxicity profile, interaction with treatments in hematological malignancies, unresolved questions and future research directions of AA therapy focusing on hematological malignancies would be discussed.

Mechanisms of action of ascorbic acid

AA was found to fight against cancers via multiple mechanisms (Figure 1). AA shows favorable features in anti-cancer treatment as a selectively cytotoxic agent as well as a targeted therapy. There are also immunomodulatory effects mediated by AA which are potentially important in immunity against cancers. The mechanisms would be discussed in detail below.

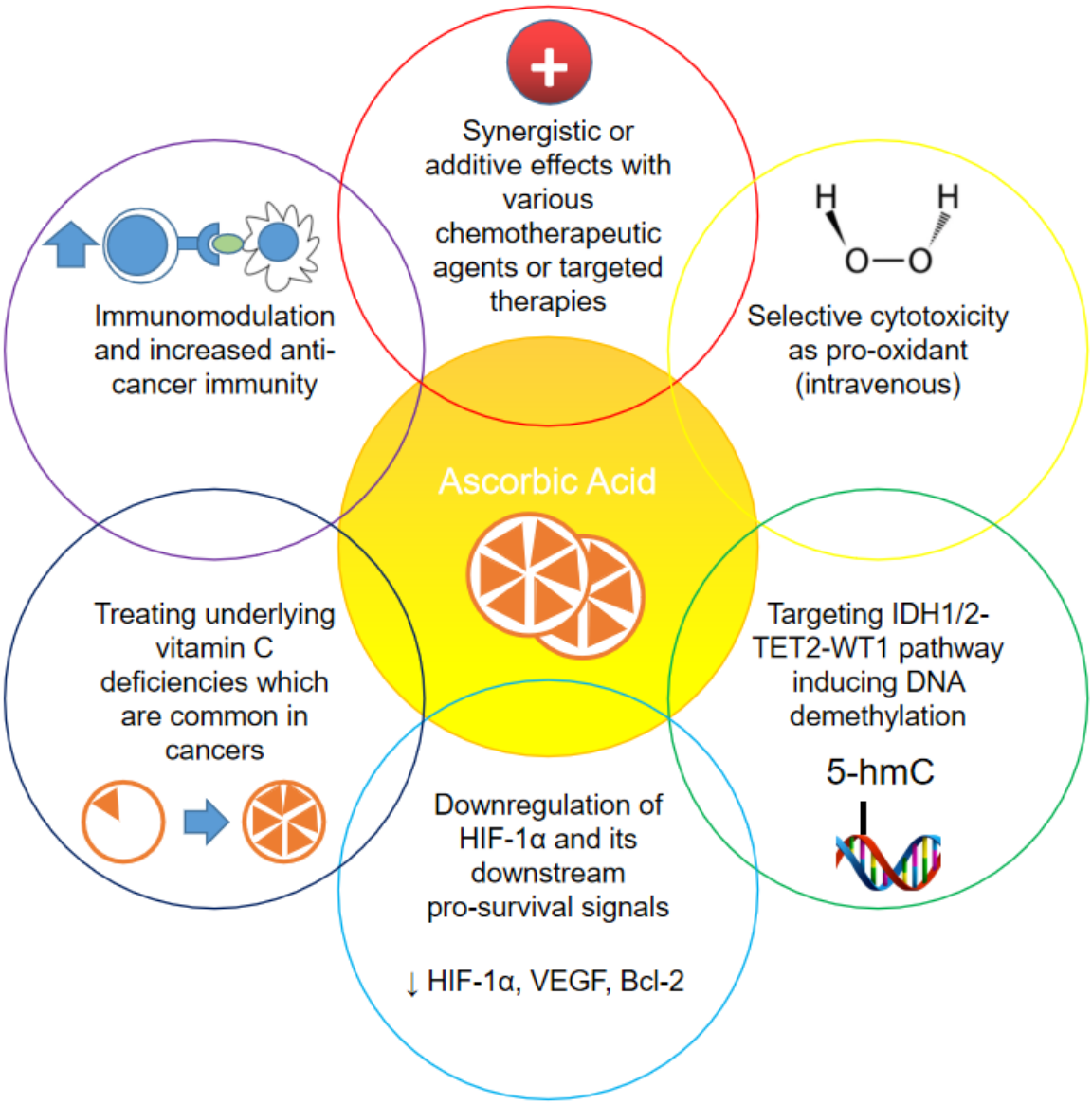

Figure 1. The major mechanisms of action of ascorbic acid against cancers. Ascorbic acid produces its anti-cancer effects via multiple mechanisms including: (i) selective cytotoxicity via reactive oxygen species generation due to its pro-oxidant properties at high concentration; (ii) targeting IDH1/2-TET2-WT1 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; (vi) synergistic or additive effects with various chemotherapeutic agents or targeted therapies.

Selective cytotoxicity of AA as pro-oxidant

AA, at high concentration, acts as 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) including hydroxyl radicals and hydrogen peroxide (H_2O_2).[8][9][Fig3] The extracellular ROS can induce cell damage by lipid peroxidation. AA enters most cells via SVCTs 1 and 2, encoded by SLC23A1 and SLC23A2 genes respectively.[10] SLC23A2 expression is much higher in hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) than in restricted hematopoietic progenitors. Its oxidized form, dehydroascorbate (DHA), enters cells via glucose transporters (GLUTs), mainly GLUT1 and

GLUT3.[9][11] Inside the tumor cells, DHA is reduced back to ascorbate at the expense of glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH).[9] Tumor cells also contain higher levels of labile iron (Fe^{2+}) than normal cells, which favor 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 is based on differential modulation of cellular responses to ROS in normal and cancer cells.[14] 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.[16] 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 high concentration of AA (average 50% lethal concentration of 3 mM). Normal cells and cord blood CD34+ HSCs have catalase activity of about 4 times higher compared with tumor cells and different leukemia cell lines.[13][17] Catalase is essential in the removal of H_2O_2, especially when H_2O_2 concentration is greater than 10 μM.[17] Kawada et al.[13] found that the apoptosis of the leukemia cell lines induced by high-dose ascorbate (≥280 μM) was almost completely abrogated by the addition of catalase. These *in vitro* study results provided insight on the reason why patients are tolerating high-dose IVAA well in general.

AA as a potential targeted therapy

Aberrant DNA methylation has been recognised as one of the important pathogenic mechanisms in epigenetic alterations in different 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 loss of function of TET2 enzyme resulting in widespread gene promoter hypermethylation (Figure 2).[18][21][22] On the other hand, TET2 mutations were also found in lymphomas, in particular 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 through its effects on TET enzymes, a family of α-ketoglutarate-dependent dioxygenases (αKGDD), which results in active DNA demethylation (Figure 2).[24] AA increases dioxygenase activity of TET2 by accelerating Fe^{3+} and Fe^{2+} redox cycle, thereby relieving some biological consequences of loss of function of TET2.[25]

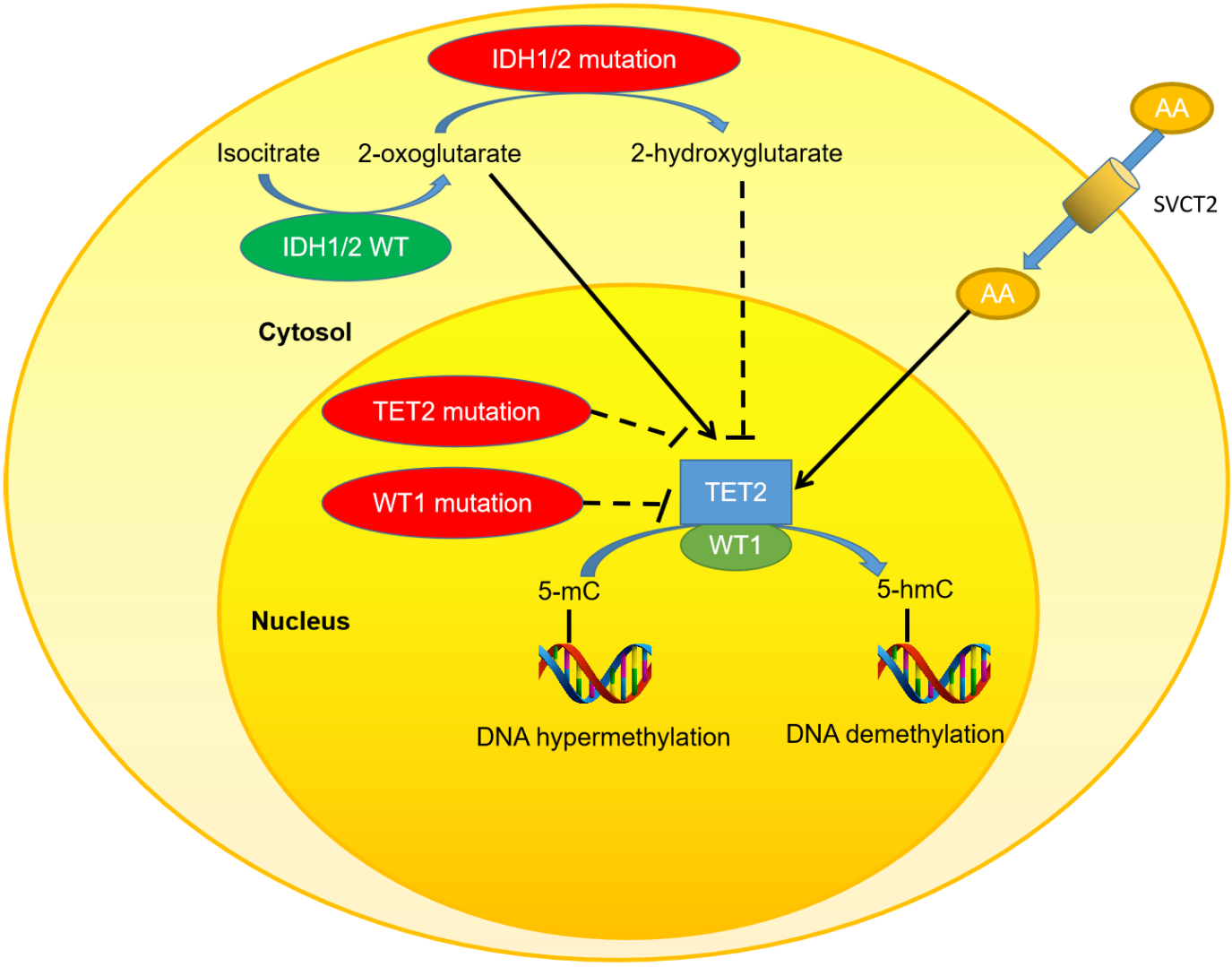

Figure 2. The mechanism of ascorbic acid on targeting the mutations in the IDH1/2-TET2-WT1 pathway. Gain-of-function mutations in IDH1 or IDH2 lead to abnormal generation of 2-hydroxyglutarate which inhibits the TET2 enzyme activity. Loss-of-function mutations in TET2 lead to decreased TET2 activity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

AA regulation of HIF hydroxylases and other αKGDD enzymes

AA was found to be a cofactor of HIF hydroxylases (another class of αKGDD enzymes).[26] HIF hydroxylases induce recognition of HIF-1α by the von Hippel-Lindau (VHL) protein with consequent ubiquitination and proteasomal degradation of HIF-1α. HIF-1α is often expressed in tumor cells and in different leukemia cell lines.[9][13] Administration of IVAA, or even just restoration of physiological level of AA by OAA supplementation, can downregulate HIF-1α and its downstream pro-survival proteins including vascular endothelial growth factor (VEGF) and Bcl-2 family of anti-apoptotic proteins.[13][26][27] Jumonji C-domain containing histone demethylases (JHDMs) are also potentially important epigenetic regulators of αKGDD class, which catalyze the histone demethylation.[9] Their role in leukemogenesis is yet to be revealed.

AA deficiencies in hematological malignancies

Humans cannot synthesize AA due to evolutionary loss of function of the enzyme L-gulono-gamma-lactone oxidase (Gulo), which catalyzes the final step in vertebrate AA biosynthesis.[28] The loss of function of Gulo is common among species with AA rich diet, therefore the mutation does not pose a selective disadvantage in general.[29] Multiple studies have also shown that serum AA levels are significantly reduced in patients with hematological malignancies.[30][31][32] This could further exacerbate the loss of function of TET2. Of note, preclinical study by Agathocleous et al.[10] have shown that supplementation with OAA was sufficient to prevent leukemogenesis when $TET2^{\Delta/+}$;$FLT3^{ITD/+}$ leukemic cells were transplanted into $Gulo^{-/-}$ mice. Restoring physiological level of AA by OAA supplementation can also downregulate HIF-1α.[27] Since physiological level of AA could be achievable by OAA supplementation, it could potentially 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.

Immunomodulatory effects of AA

Most immune cells contains high intracellular levels of AA in millimolar range, and is important for proper functioning of the immune responses.[33] Depletion of AA could lead to lower AA levels inside immune cells.[34] AA was reported to have important roles in immunity against cancer via multiple pathways involving T-helper 1 cells, cytotoxic T-cells and natural killer cells.[33]

Potential mechanisms of resistance to AA therapy

There are several preclinical studies that described potential mechanisms of resistance to AA therapy. Liu et al.[11] found that several AML and diffuse large B-cell lymphoma (DLBCL) cell lines have reduced SLC2A3 expression leading to reduced level of GLUT3, and they showed reduced response to AA. The reduction of response to AA was also demonstrated by knockdown of SLC2A3 in KG-1 AML cell line. HIF-1α overexpression and addition of catalase would also abrogate the effect of high-dose AA *in vitro*.[13] It was noted that some AML and APL cell lines show higher basal catalase activity, which could be a potential mechanism of resistance to the pro-oxidant effect of high-dose AA.[35]

Safety, adverse effect and precautions for AA therapy

High-dose IVAA and/or OAA supplementation are widely used by complementary and alternative medicine practitioners. Review on safety of IVAA and OAA showed that AA is well tolerated with no or minimal side effects in general.[36][37] Yet, there are several reported adverse effects which requires attention.

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.[38][39] In contrast, low to intermediate doses of IVAA (1-10 g every 6

hours) are safe in patients with G6PD deficiency.[40] This is coherent with the finding that pro-oxidant effect of AA predominates in high plasma AA concentration by *in vitro* studies.[13] It is recommended that G6PD screening should be done before high-dose IVAA treatment to avoid harm due to oxidative hemolysis.

Calcium oxalate calculus

AA could be metabolized to oxalate after ingestion, which could increase urine oxalate excretion and increased risk of calcium oxalate calculi formation.[41] OAA supplementation was demonstrated to be associated with mildly increased risk of renal stone formation in men but not in women.[42] High-dose AA should be used with caution in patients with history of calcium oxalate calculi, although Prier et al.[43] reported no new onset renal stone formation was noted in a prospective case series with 157 patients (8% of which have a history of renal stones) receiving IVAA during a 12-month period. Oral prophylaxis with magnesium oxide and vitamin B6 could be considered in patients with history of calcium oxalate calculi formation.[44]

Interference on glucometers leading to pseudohyperglycemia

IVAA has also been shown to interfere with many point-of-care glucose meters and this can lead to detrimental effect due to possible inappropriate insulin therapy due to the pseudohyperglycemia.[45][46] Patients are clinically well without hyperglycemic symptoms despite a high glucose reading. Clinicians should be aware of the phenomenon and be cautious on glycemic control of patients receiving IVAA. Glucometers which are resistant to such interference is preferred when IVAA is used.[47]

Antioxidants use

AA at millimolar concentrations are known to produce pro-oxidant effects. The effects could be abrogated with the use of antioxidants such as N-acetylcysteine *in vitro*.[15] Drugs with antioxidant activity should be avoided in general to prevent potential loss of pro-oxidant effects of IVAA.

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 possibility of increasing the generation of ROS and tissue damage even with OAA supplementation.[48] On the other hand, patients with iron overload may be given iron chelators for reducing iron overload. This 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][49] It is generally recommended that AA should be avoided in patients with hemochromatosis and in patients on iron chelation.

Precautions with drug administration of IVAA

There are a few precautions concerning the administration of IVAA. Riordan et al.[44] suggested that IVAA should be mixed with Ringer's lactate and/or sterile water when infused in larger amount, in a rate not exceeding 1 g of AA per

minute since the solution is hypertonic. Minerals such as calcium chloride, magnesium chloride, and potassium chloride has to be added since IVAA could cause a chloride shift which could cause hypochloremia.[50] Gradual increase in the dose of IVAA infusion is recommended, and OAA supplementation should be given when off infusion to avoid scorbutic rebound effects.[44] Discussion with pharmacists and adequate literature review is needed before starting the use of IVAA to maximize the safety of infusions.

Interaction of AA with chemotherapy, radiotherapy or targeted therapies

There were multiple preclinical *in vitro* and *in vivo* studies which demonstrated there are synergistic effects or enhanced efficacy of chemotherapy, radiotherapy or targeted therapies when high-dose AA was used in combination, although some studies on the same drug may show controversial results.[51] Moreover, the effects of different combinations may be specific to the type of tumors tested. Therefore, the effects of AA in combination with specific therapies that are commonly used and/or important in hematological malignancies are discussed in details below, based on results from preclinical or clinical studies in the field of hematology (Table 1).

Additive effect with ATO in APL

The discovery and the use of ATO in treatment of APL was a major advance in APL treatment. In the AML17 study[52], 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). Meta-analysis[53] have 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. However, the use of ATO plus ATRA is still not able to cure all patients in APL.

In vitro studies showed that high concentration of 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 which are resistant to ATRA or ATO.[15][16] High concentration of AA only shows slightly additive effect with ATO, possibly due to 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 was therefore explored. Treatment of APL with ATRA, ATO and OAA (as known as triple A therapy) was shown to have excellent outcome, with leukemia-free survival (LFS) and OS rates of 100% at 3 years and 94.1% at 5 years.[54][55] Interestingly, OAA of 1 g/day was used instead of IVAA in these patients. There is apparently improved outcome in APL using triple A therapy[54] compared with the ATRA plus ATO group in AML17 study[52], although it is not a head-to-head comparison. There may be 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] Whether using high-dose IVAA instead of OAA could produce further benefits or lower the ATO dose required is uncertain, and superiority would be difficult to be demonstrated given the excellent outcome using the current triple A therapy.

Synergy with hypomethylating agents in AML

Liu et al.[56] found that physiological concentration of AA (26-84 μM) have synergistic effect with low dose of 5-aza-2′-deoxycytidine (decitabine), a DNA methyltransferase inhibitor (DNMTi), using cancer cell lines including AML cell line (HL60). 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.[57] 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. There is no significant increase in toxicity associated with the addition of low-dose IVAA. They also showed that there is significant increase in TET2 activity by enzyme-linked immunosorbent assay (ELISA) when leukemia cell lines are treated 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).[57] A recent double-blinded randomized control trial by Gillberg et al.[32] have shown that OAA supplementation of 500 mg daily together with azacitidine treatment in patients diagnosed of either myelodysplastic syndrome (MDS), AML or chronic myelomonocytic leukemia (CMML) 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. 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, it has to be noted that global DNA demethylation with increased 5-hmC/5-mC ratio may not be predictive of superior response to treatment. Global DNA methylation analysis cannot assess the specific methylation status in the critical genes or regions of the disease. Therefore, increased 5-hmC/5-mC ratio itself does not imply a reduction in the size of abnormal clones nor a hematological response.[58]

Inhibition of proteasome inhibitors

AA was shown to inhibit bortezomib (PS-341), a proteasome inhibitor (PI) used in treatment of plasma cell myeloma, which is consistently demonstrated among different myeloma and nonhematological cancer lines and independent of its antioxidant activity.[59][60] Perrone et al.[60] showed that AA inhibits boronate PIs (including bortezomib and MG262) by direct binding, and does not abrogate the effect of other PIs including NPI0052, lactacystin or MG132. By inference, AA would likely inhibit the activity of ixazomib, which is also a boronate PI. However, the effect of AA on carfilzomib, an epoxyketone-based PI, remains uncertain. Combination of AA and PIs should be avoided in general until there is sufficient evidence that the activity of a particular PI is not inhibited by AA.

Synergy with melphalan

Melphalan is a DNA alkylator that is commonly used in treatment of multiple myeloma, either used in combination therapy for transplant-ineligible elderly patients or used in high-dose as myeloablative conditioning regimen for autologous stem cell transplantation.[61][62] Xia et al.[63] demonstrated that high-dose AA have selective toxicity against CD138+ myeloma cells *in vitro* and synergistic effect with melphalan in killing myeloma cells *in vivo*. The dose of melphalan could be

potentially reduced without losing efficacy if IVAA is added.[62]

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

Darwiche et al.[49] studied the effect of 250 μM of AA versus vehicle in combination with (i) ibrutinib (BTK inhibitor), (ii) idelalisib (PI3K inhibitor), or (iii) venetoclax (BCL2 inhibitor) using primary CLL B-cells from 40 treatment-naïve CLL patients and two CLL cell lines. They found that AA has synergistic effect with all three targeted therapies. There is also increased cell death when AA is added to fludarabine plus cyclophosphamide compared with vehicle. The potential of AA therapy in combination with CLL therapies should be further explored. On the other hand, the effect of the same combinations on other hematological malignancies may also be investigated to determine if the synergistic effect is only applicable to CLL.

Synergy with immunotherapies

There is increasing interest on AA as a potential adjunct to immunotherapies. Of note, Luchtel et al.[64] found that there is synergistic effect between anti-PD1 and high-dose intraperitoneal injection of AA leading to slower tumor growth (3-4 folds lower tumor weight) compared with vehicle, anti-PD1 or AA alone in A20 lymphoma immunodeficient mouse model. 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. Magrì et al.[65] also showed that AA enhances anti-CTLA-4 activity in mouse colorectal, breast, melanoma, and pancreatic cancer models. They also observed that the anti-cancer effect is largest when high-dose AA was administered to immunocompetent mice but not to immunocompromised mice, suggesting that AA has immunomodulatory function that requires an intact immune system to maximize its benefits.[63] On the other hand, AA has also been suggested to be potentially beneficial in chimeric antigen receptor (CAR) T-cell manufacturing process, which remains to be explored.[66]

AA and treatment-associated side effects

There are several studies which evaluated the safety of AA and the changes in symptoms related to solid tumors or treatment with chemotherapy and/or radiotherapy.[50][67][68] There are reported improvement on quality of life and reduced discomfort in cancer patients such as fatigue and pain based on 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 which could provide more objective assessment of QoL.[69]

The remaining questions to answer and directions of future research

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

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

The optimal cytotoxic effects of AA may vary among different tumor cells due to difference in sensitivity, but the usual AA level required to achieve cytotoxicity on tumor cells would be ranging from more than 250 μM to millimolar range.[13][15][16] High-dose IVAA of up to 3 g/kg in a single infusion is commonly used among CAM practitioners[70][71]. However, such high-dose infusions may not provide additional benefits, but exposes the patients to risks such as oxidative hemolysis and renal stones.[38][39][44] There is also evidence that low-dose IVAA could also produce significant benefits when used in combination with HMA and chemotherapy.[57]

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 duration commonly of 24 hours or longer.[15][49][57][60] The duration of exposure to a cytotoxic level of AA required to produce clinical benefits is uncertain. These factors should be considered to determine the optimal frequency of AA administration. Campbell et al.[26] showed that increased tumor ascorbate level could be maintained by daily administration of IVAA, even though plasma AA level would be normalized much earlier. This is associated with slower tumor growth, and reduced HIF-1α and VEGF levels, which is not seen with alternate day administration of IVAA. These findings suggest that IVAA should be given at least daily for maintaining its anti-tumor effects. This is in contrary to the usual CAM practice of IVAA administration only a few times per week.[70] Further studies including mouse models would be required to determine if more frequent low to intermediate doses of IVAA sustaining the AA concentration in millimolar range could produce similar or even superior benefits compared with high-dose but less frequent IVAA infusions.

There is also need to explore whether OAA could be used instead of IVAA in some circumstances. The use of IVAA would likely require administration in wards or day centers, but OAA could be taken at home, which is more convenient to patients. Maintaining a physiological range of plasma AA level could also potentially provide additional benefits to patients since this AA level can produce epigenetic changes and downregulate HIF-1α.[27][32] 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 managed as outpatients.

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

Das et al.[70] reported the successful use 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 IDH1/2-TET2-WT1 pathway. Yet, AA is unique as a multi-targeting agent that produces different anti-cancer effects, making addition of AA to various treatment regimens possible. The effects of adding AA in treatment of diseases with aberrations in IDH1/2-TET2-WT1 pathway versus diseases without such aberrations would be of interest. Such information could help in determining whether detection of such aberrations is predictive of better outcome with AA-containing therapy.

What combinations of treatment are of potential?

AA is shown to have synergistic or additive effects with different agents by numerous *in vitro* studies (Table 1). Further clinical trials of combining AA with such agents would be of interest. Of note, adding OAA to hypomethylating agents has

been shown to increase DNA demethylation in different myeloid cancers.[32] Further studies on the outcome of such combination is required to confirm its theoretical benefits related to increased DNA demethylation. On the other hand, there are also preclinical studies showing synergism between AA and venetoclax, idelilasib or ibrutinib in CLL.[49] Testing the combination of AA with such targeted therapies in CLL as well as in other diseases could be of value. Furthermore, combination of HMA and venetoclax is synergistic, well-tolerated and effective (CR + CR with incomplete count recovery rate of 73%) in elderly AML.[72] Since there is evidence of synergistic effects between AA and both HMA[56][57] or venetoclax[49], the combination of the three agents is of potential. The*in vitro* studies showed that the synergistic effects occur with low concentration of AA, therefore low to intermediate-dose of IVAA or high-dose OAA could potentially produce benefits[49][56][57]. On the other hand, further investigations on the addition of AA in treatment of lymphomas featured with high frequency of TET2 mutations (e.g. AITL, PTCL-NOS)[23] would be intriguing.

The aforementioned combinations are only a few examples of the many possibilities. There are already several registered phase 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)[73][74][75][76]. However, IV infusion protocols of less than once daily infusions were used in some of these clinical trials, which can potentially compromise the anti-tumor effects of IVAA based on the findings in mouse model.[26] The treatment protocols in clinical trials should be designed with regard to the pharmacokinetics and pharmacodynamics of AA to fully reveal its potential benefits.

Conclusion

AA has multiple potential benefits in treatment of hematological malignancies including selective cytotoxicity with remarkable safety, targeting of IDH1/2-TET2-WT1 pathway, correction of vitamin C deficiencies which are common in hematological malignancies, producing synergistic effects with numerous chemotherapeutic agents, radiotherapy and targeted therapies. Unlike other novel targeted therapies, AA is much more affordable to patients. The potential of treatment using AA in combinations of different current standard therapies 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. [56]
	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. [57]
Bortezomib	5-10 nM	MM	*in vitro*	6 cell lines	AA of 30-500 mM for 24 hours	Inhibitory ↑ tumor growth	Perrone et al. [60]
	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. [63]
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. [49]
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. [64]

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 [73]	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 [74]	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 [75]	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 [76]	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

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; VAF, variant allele frequency

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