Parenteral high-dose ascorbate - A possible approach for the treatment of glioblastoma (Review)

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Abstract. For glioblastoma, the treatment with standard of care therapy comprising resection, radiation, and temozolomide results in overall survival of approximately 14-18 months after initial diagnosis. Even though several new therapy approaches are under investigation, it is difficult to achieve life prolongation and/or improvement of patient's quality of life. The aggressiveness and progression of glioblastoma is initially orchestrated by the biological complexity of its genetic phenotype and ability to respond to cancer therapy via changing its molecular patterns, thereby developing resistance. Recent clinical studies of pharmacological ascorbate have demonstrated its safety and potential efficacy in different cancer entities regarding patient's quality of life and prolongation of survival. In this review article, the actual glioblastoma treatment possibilities are summarized, the evidence for pharmacological ascorbate in glioblastoma treatment is examined and questions are posed to identify current gaps of knowledge regarding accessibility of ascorbate to the tumor area. Experiments with glioblastoma cell lines and tumor xenografts have demonstrated that high-dose ascorbate induces cytotoxicity and oxidative stress largely selectively in malignant cells compared to normal cells suggesting ascorbate as a potential therapeutic agent. Further investigations in larger cohorts and randomized placebo-controlled trials should be performed to confirm these findings as well as to improve delivery strategies to the brain, through the inherent barriers and ultimately to the malignant cells.

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Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; AQPs, aquaporins; AA, ascorbic acid; ATM, ataxia telangiectasia mutated; BBB, blood-brain-barrier; CSF, blood-cerebral spinal fluid; bw, body weight; CNS, central nervous system; CAR, chimeric antigen receptor; CP, choroid plexus; DHA, dehydroascorbate; EGFR, epidermal growth factor receptor; ECF, extracellular fluid; GLUT1, glucose transporter 1; H_2O_2, hydrogen peroxide; HIFs, hypoxia inducible factors; i.v., intravenously; IDH, isocitrate dehydrogenase, GULO, L-gulono-γ-lactone-oxidase; mTOR, mammalian target of rapamycin; MGMT, O^6-methylguanine-DNA methyl-transferase; SVCTI, sodium-dependent vitamin C transporter; SLC2A21, solute carrier family 2, facilitated glucose transporter, member 1; TERT, telomerase reverse transcriptase; TET, ten-eleven translocation; WHO, World Health Organization

Key words: glioma, glioblastoma, high-dose ascorbate, vitamin C, temozolomide, astrocytes

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1. Introduction

Cancers of the central nervous system (CNS) affect the brain in 95% of cases and the remaining 5% are distributed among the meninges and spinal cord skin, cranial nerves, and spinal cord. Initially, tumors of CNS were classified based on their tissue and cell origin. Gliomas are of neuroectodermal origin, derived from the supporting glial cells within the CNS (1). They demonstrate a considerable variability in age of onset, grade of severity, histological features, and ability to progress as well as to metastasize (1-5).

According to the classification of CNS tumors published by the World Health Organization (WHO) in 2016, glioblastoma belong to the group of diffuse astrocytic and oligodendroglial

tumors with aggressive clinical behavior corresponding to WHO grade IV comprising three subgroups: Primary glioblastoma, secondary glioblastoma with and without presence of isocitrate dehydrogenase (IDH) mutations, and the third one containing not otherwise specified glioblastoma tumors (1). Glioblastoma with IDH wild type represents 90% of the cases, which most frequently corresponds to the clinically defined primary or *de novo* glioblastoma that is predominantly found in patients aged over 55 years (6). Secondary glioblastoma with a history of prior lower-grade diffuse glioma mainly arises in younger patients and presents approximately 10% of the cases. The highest incidence with ~65% is reported for individuals aged more than 65 years with the average annual age-adjusted rate of approximately 3 per 100,000 individuals per year (7). Glioblastomas are 1.58-fold more prevalent in males than in females. With a median survival time of 14 months, the diagnosis is very poor for patients with WHO grade IV tumors and only 5.6% of patients survive longer than five years post diagnosis (7-10).

The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy concluded that histologic grade II and III IDH wild-type diffuse astrocytic gliomas that contain a high-level of epidermal growth factor receptor (EGFR) amplification, a combination of whole chromosome 7 gain and whole chromosome 10 loss (+7/-10), or telomerase reverse transcriptase (TERT) promoter mutations, correspond to WHO grade IV and should be referred to as diffuse astrocytic glioma, IDH wild type, with molecular features of glioblastoma, WHO grade IV (11). It was also suggested that specific molecular signatures in subsets of IDH wild-type diffuse astrocytic gliomas are associated with better clinical outcomes and should not lead to a high-grade designation including those gliomas with other genetic alterations as individual drivers (11).

In order to accomplish early diagnosis and to develop personalized anti-cancer therapy, studies have focused on the identification of valid tumor markers that are easily accessible, can be simply analyzed, and provide accurate information regarding disease and severity (12). Epigenetic dysregulation of the ten-eleven translocation (TET) enzymes results in frequent epigenetic alterations in human glioblastoma including DNA hypermethylation and hypo-hydroxymethylation, as well as loss of histone acetylation (13).

Among others, the methylation status of the O^6-methylguanine-DNA methyl-transferase (MGMT) gene promoter is such a molecular biomarker (14). Further genetic parameters, small non-coding RNAs, proteins, other small molecules, circulating tumor cells, and extracellular vesicles are possible candidates under investigation. These markers are quantifiable in tumor tissue or in body fluids such as blood, urine, or central spinal fluid (2). However, an established and generally accepted guideline to using them for diagnostic purposes is not available at the moment.

One of the current treatment options includes surgical resection aiming to remove as much contrast-enhancing tissue as possible without giving the patient a new functional deficit (15-17). Since most recurrences occur in close proximity to the primary tumor or in the tumor bed, percutaneous fractionated radiotherapy should also be performed (18). At present, the standard chemotherapeutic drug for glioblastoma is temozolomide (19-22).

Despite the aggressive therapy and research efforts, the prognosis for survival remains extremely poor and has not improved much over the last decades. The chemotherapeutic options are limited and restricted by poor distribution in the CNS due to the infiltrative nature, prominent angiogenesis, and vascularization of glioblastoma as well as acute systemic toxic effects and long-term toxicity in the CNS and bone marrow. The current research is dominated by checkpoint inhibitor trials, vaccine trials, and gene therapies (23). Of note, tumor-treating fields are electric fields that represent a non-invasive cancer treatment option that is applied locally, displaying good results in addition to standard of care therapy to improve the survival of newly diagnosed patients (24).

There has also been some success via the application of oncolytic viruses in the treatment of glioblastoma such as H1-parvovirus (25). The effects were caused in part by direct oncolysis but also by inducing the antitumoral immune response (25). Dendritic cell or peptide immunotherapy and chimeric antigen receptor (CAR) T-cell therapy are also under consideration (26). Even though there are some promising novel approaches, there remains a need to improve glioblastoma treatment options.

Interestingly, high vitamin C or ascorbate concentrations were shown to damage cancer cells by exhibiting pro-oxidative effects. On the other hand, high-dose ascorbate seems to be well tolerated by non-malignant cells (26-29). The aim of this review was to examine the current findings in regard to the therapeutic potential of pharmacological doses of vitamin C in the treatment of glioblastoma.

For this review, the literature research was performed using key words including 'glioblastoma', 'glioma', 'glioblastoma multiforme', 'vitamin C', 'ascorbate', 'ascorbic acid', and 'pharmacological' 'intravenous', 'IV', and 'high-dose' 'blood brain barrier', 'brain', 'cancer', 'vitamin C or ascorbate transporter', 'clinical trials', 'treatment', 'human' as well as 'ascorbate derivatives'. The terms were entered solely or in combination to find original articles and reviews on the homepage of the National Library of Medicine (PubMed.gov). The search was not restricted to publication date or other specifications, even though results from newly published studies were preferred over the older ones. However, in case of differences, both were cited in general. If results from clinical trials were published as original research, the details from human studies were verified using the following data sources https://clinicaltrials.gov, https://www.clinicaltrialsregister.eu/ctr-search/search, and https://www.who.int/clinical-trials-registry-platform.

2. Vitamin C

Vitamin C, also known as L-ascorbic acid (AA) or ascorbate, is an essential micronutrient and its deficiency is associated with several serious symptoms and ultimately death (30). In humans and most primates, vitamin C needs to be supplied by the diet due to the lack of functional enzyme L-gulono-γ-lactone oxidase (GULO), which catalyzes the last step of AA biosynthesis (31). In fluids, vitamin C occurs in two major forms as ascorbate (90%) or in its oxidized form dehydroascorbate (DHA). Under physiological conditions, the amount of DHA in plasma is estimated as <1-2% relative to plasma ascorbate levels (32). The recommended daily dose of vitamin C varies

between 75 and 100 mg resulting in physiological plasma levels of 50-100 μmol/l (33,34). However, there are major discrepancies regarding recommendations for dietary vitamin C intake depending on individual physiological and pathophysiological conditions (35).

With ingested amounts found in foods, vitamin C plasma concentrations usually do not exceed 100 μmol/l (32). Even after oral supplementation, ascorbate plasma concentrations stay below 250 μmol/l and often lower than 150 μmol/l (32,36). By contrast, after intravenous ascorbate injection (i.v.), pharmacologic plasma ascorbate concentrations of 26.2 +/-4.9 mmol/l are safely achieved (37). Serious side effects were only reported for patients with pre-existing renal insufficiency or glucose 6-phosphate dehydrogenase deficiency, both known to be predisposed to vitamin C toxicity (38). Besides tissue accumulation and renal reabsorption, the major determinant of plasma concentration of orally ingested vitamin C is the saturable capacity of the gut. Parenteral ascorbate bypasses the intestinal absorption mechanisms that are responsible for this limitation and therefore allows the use of ascorbate as a pharmacological agent. For vitamin C, a linear relationship between dose and C_{max} can be observed up to 70 g/m^2 in humans [approximately 112 g in females (body surface: 1.60 m^2) and 133 g in males (body surface: 1.90 m^2)] as complied from pharmacokinetic studies, while higher doses do not translate into higher plasma C_{max} levels (39,40).

There is growing evidence that patients with cancer have lower vitamin C plasma levels than healthy controls (41-46) and a large proportion of them exhibit hypovitaminosis for vitamin C or manifest deficiency (47-53). Severity of the disease also appears to correlate with the vitamin C status and higher stages of cancers seem to be associated with lower vitamin C levels (52,54,55). Mayland *et al* showed that patients with low vitamin C plasma levels have a significantly worse prognosis than patients with sufficient plasma levels (48). Although it is suspected that vitamin C blood levels are low in patients with glioblastoma, no data according vitamin C deficiency, symptoms, and disease progress are currently available for this tumor entity.

3. Vitamin C metabolism in normal CNS tissue

Ascorbate is required for homeostasis and proper functioning of the central nervous system. The brain consumes a large portion of glucose (~25%) and oxygen (~20%), which implies rapid metabolism with increased free radical production. Being an organ that metabolizes oxygen with relatively weak protective antioxidant mechanisms, the brain is particularly susceptible to oxidative stress (56). It therefore depends on high levels of antioxidants to maintain redox balance. Accordingly, ascorbate is the physiologically most abundant antioxidant present in brain tissue.

As a water soluble agent, vitamin C is absorbed from the small intestine and then distributed from blood throughout the extracellular space (32). Vitamin C is accumulated in tissues against a concentration gradient. The concentrations of vitamin C in tissues are frequently higher (up to 4,000 μmol/l) than in fluids (up to 300 μmol/l) and may simply serve as a reservoir or have other unknown functions (32,36,57). It should be noted that for many human tissues accurate vitamin C concentrations are not known for physiological nor pathophysiological conditions. The total average concentration of vitamin C in the brain is lower in comparison to the adrenal gland, lens, or liver. Within the brain, the highest concentration was observed in the pituitary gland and accumulation in neurons with 10 mmol/l was markedly higher than in the cerebral spinal fluid or glial cells with only 1 mmol/l suggesting an important role in the maintenance of neuronal integrity (32,58-61).

4. Entrance and distribution of vitamin C in the brain

As a hydrophilic molecule, vitamin C enters the CNS from the blood-brain-barrier (BBB) or from the blood-cerebral spinal fluid (CSF) barrier formed by epithelial cells of the choroid plexus (CP) (Fig. 1) (62,63). Vitamin C initially accumulates in CP cells and then passes through the CSF into the brain (63). This observation confirms the data from knockout mice analysis and cell models (64-68).

The incorporation of ascorbate in the cells is mediated via specific transporters termed sodium-dependent vitamin C transporter (SVCT) 1 and 2 (69-72). SVCT2 is the absorptive vitamin C transporter in the brain (69). DHA is translocated via the solute carrier family 2, facilitated glucose transporter, member 1 [SLC2A1 or glucose transporter 1 (GLUT1)] (73). Due to the lack of SVCT2 in cerebrovascular endothelial cells, transport via BBB occurs as DHA via GLUT1 (74). As DHA concentration is low in blood, the role and influence of this transport route into the extracellular space regarding the maintenance of the ascorbate concentration in the brain seems to be insignificant under physiological conditions.

Ascorbate enters CNS through SVCT2 present in choroid plexus cells on the basal side (75,76) of the CP and also probably through GLUT1 (69,77) and is then distributed into ventricular space from which it penetrates across ependyma and pia mater deeply into the brain (78). The concentration of ascorbate is thereupon balanced between cerebrospinal fluid and the extracellular fluid (ECF) by diffusion through ependymal cells.

SVCT2 is not expressed in astrocytes under physiological conditions but these brain cells play an important role in the regeneration of DHA into ascorbate. It is postulated that astrocytes incorporate DHA through GLUT1 (79,80) and then convert DHA into ascorbate due to the 4-fold higher intracellular glutathione levels compared to neurons (81). After the conversion of DHA to ascorbate within astrocytes, it is released from astrocytes and extracellular ascorbate is capable of entering neurons via SVCT2 again (82). This circuit describes the bystander effect including vitamin C recycling between astrocytes and neurons, in which neurons are the 'activated cells' inducing oxidation of ascorbate to DHA which is subsequently absorbed by the astrocytes as 'bystander cells' (63,83).

This ascorbate recycling as an interaction between astrocytes and neurons is crucial for the maintenance of normal brain ascorbate levels required for different functions inside the CNS such as catecholamine biosynthesis (84), peptide amidation (85), myelin formation (86), enhancement of synaptic activity (87), protection against glutamate toxicity (88), and modulation of precursor cell proliferation and differentiation (63,89,90). Taken together, vitamin C is

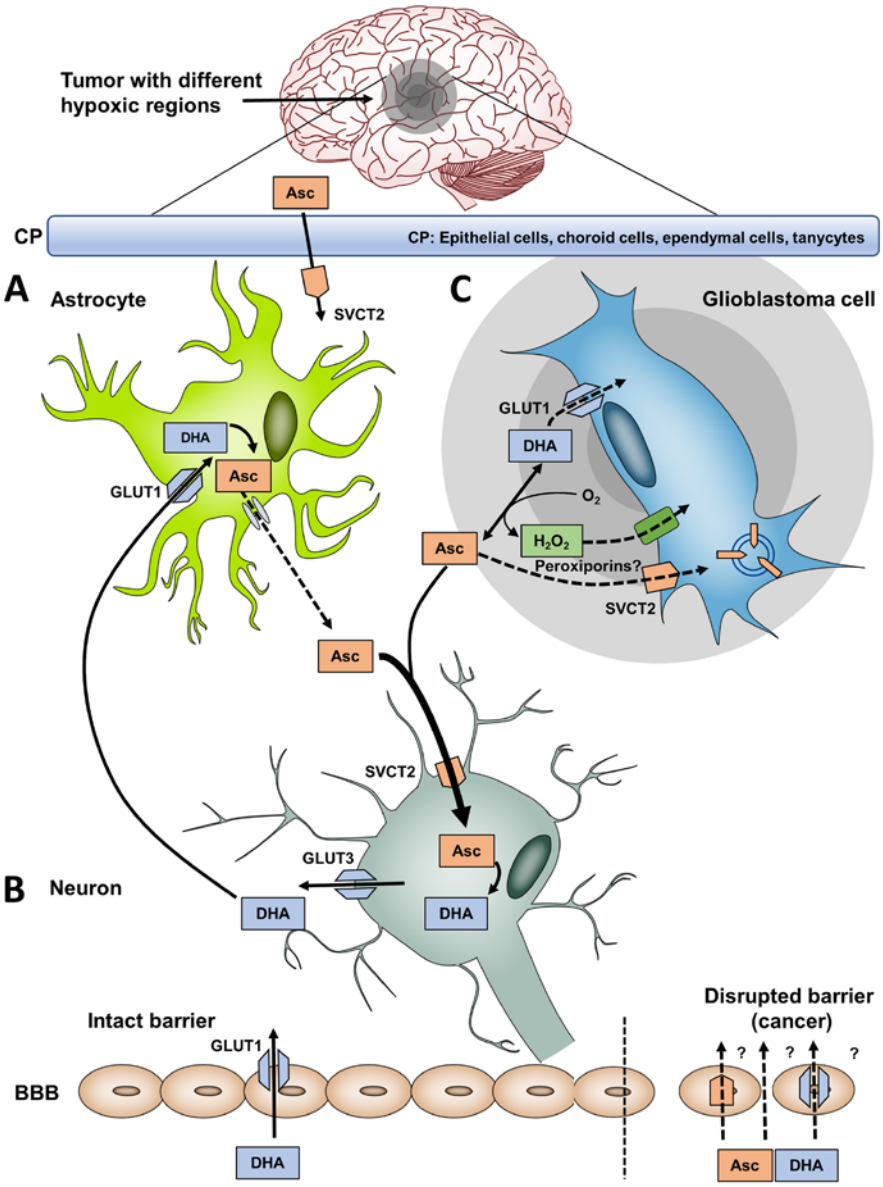

Figure 1. Schematic representation of vitamin C uptake and compartmentalization in the brain with resident glioblastoma. Vitamin C (Ascorbate=Asc) enters the central nervous system (CNS) by two routes, via blood-brain-barrier (BBB, lower layer of cells) and blood-central-spinal fluid-barrier (blue horizontal bar at the top, above the cell images), BBB and choroid plexus (CP) represent a tightly regulated barrier for precise substance entrance into the brain. Vitamin C translocates from the blood through BBB in its oxidized form as dehydroascorbate (DHA) using glucose transporter 1 (GLUT1). CP is formed by epithelial cells, choroid cells, and ependymal cells or tanycytes as mentioned within the blue bar. The entry of vitamin C in its reduced form is mediated by sodium-dependent vitamin C transporter 2 (SVCT2) expressed within the choroid cells. Under pathophysiological conditions such as glioblastoma, the barrier seems to be disrupted. Thus, the vitamin C transporters expression and their functionality may be altered as the total amount of vitamin C entering the nervous system is changed. Dashed lines with arrows represent processes, which are still unknown or the hierarchy of three members is not finally understood. Continuous lines with arrows represent known routes according to the current state of knowledge. (A) The major substrate for astrocytes is DHA, which enters into the cell via GLUT1. After regeneration, vitamin C leaves astrocytes and is either usable extracellularly or for neurons. (B) Once inside CNS, vitamin C is consumed by neurons and utilized to DHA. DHA is then released by neurons via GLUT3 into the extracellular space. (C) The expression patterns and the functionality of vitamin C transporters (SVTC2 and GLUT1) as well as their role in the cellular uptake of vitamin C are still not fully clarified. Furthermore, vitamin C is able to produce hydrogen peroxide (H_2O_2) within the extracellular space. Peroxiporins, as a subtype of aquaporins, are speculated to be the possible channels for H_2O_2 entrance into the cancer cell. Adapted from (28,63,73,77,176,179,180,189).

found in higher concentrations in CSF and brain parenchyma (200-400 μmol/l) than in plasma (30-60 μmol/l) (91,92).

5. Vitamin C in cancer

Since Cameron and Pauling demonstrated that ascorbate-treated terminal cancer patients experienced a 4.2-fold increase in the mean survival time compared to untreated controls (93), the role of vitamin C in tumorigenesis and anti-cancer treatment has become the object of investigations and still not finally clarified. Twenty years later, the pharmacokinetics of ascorbate were described by Levine *et al* (36).

Ascorbate was an early unconventional and inexpensive therapy for the treatment of cancer with an excellent safety profile and surprising clinical efficacy (93). However, as two clinical trials with oral ascorbate failed to reproduce these effects, ascorbate was not used any more in the conventional oncologic therapy and shifted to the field of complementary

and alternative medicine (94,95). Interestingly, previous studies resulted in the re-examination of ascorbate treatment and it became clear that only parenteral application of ascorbate yielded millimolar plasma levels with high efficacy against tumor progression (96-99). As the existing evidences are preliminary, there is a number of fundamental questions around best clinical practice, frequency of therapy, dosage, duration as well as treatment guidelines for each tumor entity and grade (100-102).

The chemistry of ascorbate defines its biological activity in terms of its function as an anti-oxidant as well as a pro-oxidant. It initiates selective toxicity in cancer cells (26) but not in normal cells and enhances cytotoxic effects in combination with radiotherapy and/or chemotherapy (103). The prodrug effect of pharmacological ascorbate includes its ability to form and deliver extracellular hydrogen peroxide (H_2O_2) to tissue (27). H_2O_2 crosses cell membranes via peroxiporins (104-106). The selective toxicity of ascorbate depends on an altered H_2O_2 metabolism in cancer compared to non-malignant cells.

The mechanism of cytotoxicity of pharmacological vitamin C is not only associated with high levels of extracellular H_2O_2 (26) but also involves labile iron pools. These catalyze the oxidation of ascorbate to produce H_2O_2 thereby generating the hydroxyl radical from H_2O_2 via the Fenton reaction and causing oxidative damage to cellular lipids, proteins, and DNA (Fig. 2) (28). This effect is well described for neuroblastoma (107). Therefore, there are two mechanisms, which contribute to the selective toxicity of ascorbate in cancers: First, a reduced capacity to remove H_2O_2 and second, superoxide as well as H_2O_2-induced disruption of the iron metabolism with increased levels of redox active iron (28,103,108-111). Interestingly, the addition of extracellular catalase *in vitro* eliminates the anti-tumoral effects of high-dose ascorbate almost completely and therefore formation of extracellular H_2O_2 (112,113) seems to be a major contributor to the anti-tumoral effects. The intracellular reactive oxygen species formation appears to also affect cancer cells containing large amounts of labile iron (28). Furthermore, elevated iron levels identified in cancer cells activate iron-dependent proteins that promote adaptation to hypoxia and stimulate cell proliferation (114,115). The question whether ascorbate or DHA is the most effective vitamin species was elucidated in cell models (28,116,117) and showed that GLUT-mediated DHA uptake does not play a major role in ascorbate toxicity. This is biologically plausible, because DHA cannot form toxic H_2O_2, extracellularly.

The concentration of H_2O_2 seems to determine the expression and activity of the antioxidant enzymes that remove H_2O_2, including catalase, glutathione peroxidase, and peroxiredoxins, whereas catalase levels were shown to be lower in malignant-transformed cells compared to untransformed ones (111,118,119).

The metabolism of tumor cells differs from that of normal cells with a shift from energy-producing pathways to those generating macromolecules necessary for proliferation and tumor growth, known as the Warburg effect. This is evident together with hypoxia, a characteristic sign of solid tumors (120,121). Several mechanisms are involved in the development of hypoxia causing significant heterogeneity in the tissue oxygen levels in tumors (122). Adapting to an oxygen-depleted microenvironment, tumors upregulate hypoxia inducible factors (HIFs) and shift to an anaerobic energy production (123). Previous studies in patients with brain cancer have already described an increase of the expression of HIF-1 and GLUT-1 correlating to the malignancy grade (from grades II to IV) (124-126). Previous findings on glioblastoma cells showed a novel epigenetic mechanism underlying modulation of HIF-1 transcriptional activity that enable cancer cells to rapidly respond to hypoxic stress (127). A hypoxia-induced negative feedback mechanism that maintains high activity of HIF-1 and cell mobility in human glioblastoma cells has been suggested (112,127). Previous findings on breast cancer tissue showed that higher vitamin C concentrations in tumor tissue correlate with lower HIF-1 activity and increased disease-free and disease-specific survival (128).

Importantly, ascorbate-dependent restoration seems to play a role in cancer epigenetics. Given the fact that ectopic overexpression of *TET2* regulates neural differentiation in glioblastoma cell lines and impairs tumor growth (13), it would be meaningful to assess the effects of vitamin C in this process (129). Results of a recent randomized clinical trial focusing on intravenous vitamin C, adjuvant to decitabine, showed activation of *TET2* in leukemic cells and a significant improvement in overall survival in elderly patients with acute myeloid leukemia (12).

Alteration in the differentiation potential of cancer stem cells and blocking metastasis is also important (130). Experiments with different glioblastoma cells showed that changes of mitochondrial oxidative and intracellular iron metabolism in combination with pharmacological ascorbate treatment induces cancer cell selective toxicity (28). Moreover, daily pharmacologic ascorbate treatment significantly decreased growth rates of gastric, ovarian, and pancreatic cancers as well as glioblastoma established in mice (99,131-134). There are numerous studies demonstrating the usage of pharmacological ascorbate as an adjuvant to enhance radiation or chemotherapy responses (113,131,135-138). Millimolar concentrations of ascorbate were able to induce pro-oxidative effects in the interstitial fluid of the tumor cells mainly caused by extracellular H_2O_2 formation resulting in DNA damage in tumor cells and cellular adenosine triphosphate (ATP) depletion. Furthermore, it was described that the ataxia telangiectasia-mutated (ATM)/adenosine monophosphate-activated protein kinase (AMPK) pathway was activated and the mammalian target of rapamycin (mTOR) was inhibited in ovarian cancer cells (99). The combination of parenteral ascorbate with the conventional chemotherapeutics carboplatin and paclitaxel synergistically inhibited the growth of ovarian cancer in mouse models and reduced chemotherapy-associated toxicity in patients with ovarian cancer by ascorbate treatment (99).

In accordance with the data from *in vitro* and rodent models, in which pharmacologic concentrations of ascorbate act as a pro-oxidant locally on cancer cells and as an antioxidant preventing normal tissue from chemotherapy or radiation injury, ascorbate was applied intravenously to humans in clinical trials. It is generally challenging to transfer *in vitro* results to the *in vivo* situation, especially regarding glioblastoma treatment, since high-dose ascorbate has to pass the BBB and other tumor-related conditions. The interaction of

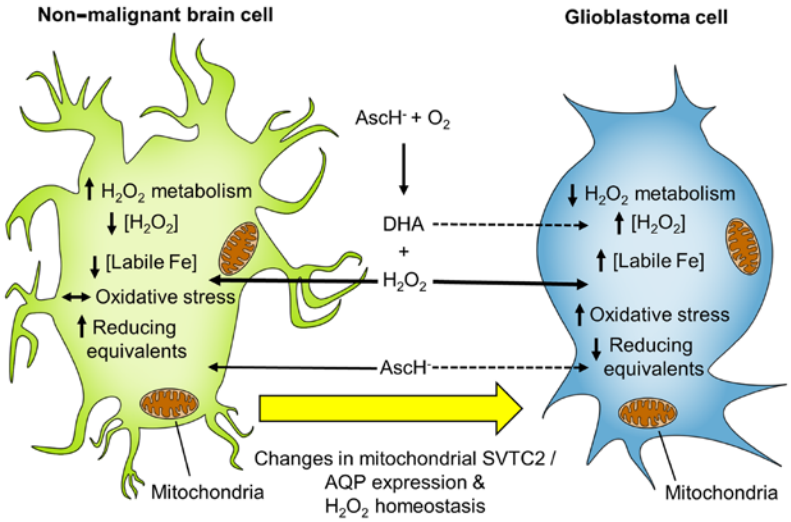

Figure 2. Differences between non-malignant and glioblastoma brain cells in hydrogen peroxide (H_2O_2) metabolism and redox-active iron metabolism (Fe) induced by pharmacological ascorbate ($AscH^-$). The selective toxicity of pharmacological ascorbate to cancer cells is mainly an H_2O_2-mediated mechanism. Extracellular oxidation of ascorbate produces H_2O_2, which channels into the cell. In non-malignant cells, vitamin C is non-toxic due to a high capacity to metabolize H_2O_2 in relation to well-regulated iron metabolism. These properties limit the levels of redox-active, labile iron, and the associated production of oxidizing free radicals. The absence of ascorbate-mediated oxidative distress allows the reduction of ascorbate capabilities as an antioxidant. By contrast, decreased capacity of cancer cells to remove H_2O_2 as well as cancer-cell-specific disruptions in iron metabolism result in increased levels of labile iron and lead to significant oxidative stress as well as to decreased amounts of reducing equivalents. Both tumor-determined cellular alterations and chemo-radiation-induced free radical production cause an increased susceptibility of glioblastoma cells to pharmacological ascorbate compared to non-malignant cells. These changes include vitamin C, its metabolites, and H_2O_2 transport capabilities through the cellular and mitochondrial membrane, via sodium-dependent vitamin C transporter 2 (SVCT2) and peroxiporins as well as their altered metabolism. Dashed lines represent mechanisms, whose role in the overall vitamin C actions is not finally clarified. Continuous lines with arrows represent known routes according to the current state of knowledge. Adapted from (27,28,103,180).

neurons and astrocytes is also crucial, e.g., the regeneration of ascorbate from DHA. In addition, a considerable number of clinical studies showed that cancer patients treated with intravenous ascorbate in addition to chemotherapy or radiation demonstrated a significantly ameliorated performance whereas therapy-induced side effects were limited, resulting in improved quality of life (37,99,139,140).

Nauman *et al* performed a systematic review of intravenous ascorbate in clinical cancer studies. A total of 23 clinical trials (all of phase I/II) were examined, therefrom 11 trials were ongoing and 11 studies aimed to evaluate low-dose intravenous ascorbate with arsenic oxide (141). The included clinical trials from different cancer entities were aimed to evaluate the effects of intravenous ascorbate.

Overall, it was evident that ascorbate was safe in almost all patient populations and exhibited potential to reduce toxicities of other cancer treatment regimens. The chemotherapeutic potential of vitamin C requires high concentrations, which can only be achieved intravenously. However, the IC_{50} values of vitamin C against tumor cells determined *in vitro* vary greatly depending on the tumor cell line employed. No conclusive clinical data are yet available regarding the necessary intravenous amount. Most studies use a frequency of at least twice weekly and doses of approximately 1 g per kg body weight (bw) with promising results in solid tumors (96,97,141). One randomized controlled clinical trial observed benefits with smaller amounts of 60-80 mg/kg bw in patients with acute myeloid leukemia (12). However, a small trial with doses lower than 1 g/kg seemed to be therapeutically ineffective (142). Considering different cancer entities, applied chemotherapeutic agents and regimens of radiation, the goal of most investigations was to achieve a plasma concentration of approximately 22 mmol/l as described by Riordan *et al* (49). The therapeutic concentration and frequency in high-dose ascorbate studies differed from 50 to 100 g and higher per day as infusion, twice to three times weekly and the duration of treatment depended on study design (141-145).

6. Trials evaluating pharmacological ascorbate in glioblastoma

In addition to an overview on the clinical use of vitamin C and its pharmacokinetics with an emphasis on bioavailability in the CNS, this review focused also on the study situation on high-dose intravenous ascorbate in oncology with particular relevance to glioblastoma. Using the terms 'glioblastoma', 'glioma', and 'glioblastoma multiforme' as well as 'vitamin C', 'ascorbate', 'ascorbic acid', and 'pharmacological' 'intravenous', 'IV', and 'high-dose' in the following data sources https://clinicaltrials.gov, https://www.clinicaltrialsregister.eu/ctr-search/search, https://www.who.int/clinical-trials-registry-platform, and https://www.ncbi for clinical trials, a total of 11 studies were identified (Table I). Therefrom, intravenous ascorbate was found to be applied in four phase I and II trials.

In the Phase I trial NCT01752491 (active, not recruiting), the safety of high-dose ascorbate was tested in combination with chemotherapy and temozolomide according to Stupp *et al* (8) in a total of 13 study subjects. Only patients above 18 years of age were included in that study. Thereby, dose escalation (15-125 g) was performed for the radiation and for the adjuvant periods for each subsequent study subject. Escalating weekly doses of ascorbate (up to 125 g) to target a serum level of 350 mg/dl (20 mM) showed that application

Table I. Overview of *in vivo* studies with human glioma and neuroblastoma patients involving ascorbate application.

Study title	Intervention	Route/dosage (ascorbate)	Age	Enrollment	Study status (phase)/outcome	Sponsor/collaborators	NCT number
A phase I trial of high-dose ascorbate in glioblastoma multiforme	Drug: Temozolomide Radiation: Radiation therapy Drug: Ascorbic acid	Intravenous infusion, 15, 25, 50, 62.5, 75 to 87.5 g of ascorbate/ infusion	18 years and older (adult, older adult)	n=13 (a)	Active (I), not recruiting, estimated completion date: December 2021, study with results: Tumor suppressive, life prolongation, reducing adverse events improving patient's quality of life	Joseph J. Cullen, MD, FACS National Institutes of Health (NIH) National Cancer Institute (NCI) University of Iowa (146)	NCT01752491
A phase II trial of high-dose ascorbate in glioblastoma multiforme	Drug: Temozolomide Radiation: Radiation therapy Drug: Ascorbic acid	Intravenous infusion, twice or three times per week, 87.5 g of ascorbate/ infusion	18 years and older (adult, older adult)	n=90 (e)	Active (II), not recruiting, estimated completion date: December 2024, follow-up to NCT011752491, results not yet available	Bryan G. Allen, MD, PhD Holden Comprehensive Cancer Center National Cancer Institute (NCI) University of Iowa	NCT02344355
A phase I study of high-dose l-methylfolate in combination with temozolomide and bevacizumab in recurrent high-grade glioma	Drug: Bevacizumab Drug: Temozolomide Dietary supplement: Vitamin C	Oral administration, 250 mg vitamin C once a day as dietary supplement	18 years and older (adult, older adult)	n=12 (a)	Active (I), not recruiting, estimated completion date: January 2022, results not yet available	Stephen Clark, MD Vanderbilt-Ingram Cancer Center	NCT01891747
Temozolomide and ascorbic acid in treating patients with recurrent high-grade glioma	Dietary supplement: Ascorbic acid Drug: Temozolomide Other: Quality-of-life assessment Other: Laboratory biomarker analysis	Intravenous infusion, three times per week (every 4 weeks for up to 12 courses) maximum tolerated dose of ascorbic acid in combination with temozolomide	19 years and older (adult, older adult)	n=4	Terminated (I), completion date: August 2015, lack of efficacy	Nicole Shonka, Principal Investigator University of Nebraska National Cancer Institute (NCI)	NCT02168270
Multimodal molecular targeted therapy to treat relapsed or refractory high-risk neuroblastoma	Drug: Dasatinib Drug: Rapamycin (excipient palmitoyl ascorbic acid among others) Drug: Irinotecan Drug: Temozolomide	Oral administration, rapamycin (excipient palmitoyl ascorbic acid)	Up to 25 years (child, adult)	n=130	Terminated (II), completion date: September 2020, ascorbic acid used as excipient	Selim Corbacioglu, MD Principal Investigator University of Regensburg	NCT01467986

Table I. Continued.

Study title	Intervention	Route/dosage (ascorbate)	Age	Enrollment	Study status (phase)/outcome	Sponsor/collaborators	NCT number
Bevacizumab and ascorbic acid in patients treating with recurrent high-grade glioma	Dietary supplement: Ascorbic acid Biological: Bevacizumab Other: Laboratory biomarker analysis Other: Quality-of-life assessment	Intravenous infusion, three times per week (every 4 weeks for up to 12 courses)	19 years and older (adult, older adult)	n=9	Terminated (I), completion date: March 2019, not available	Nicole Shonka, Principal Investigator University of Nebraska National Cancer Institute (NCI)	NCT02833701
Potentiation of chemotherapy in brain tumors by Zinc	Dietary supplement: zinc and ascorbate	Oral administration, Zinc and ascorbate	18 years to 90 years (adult, older adult)	n=30 (e)	Recruiting (not applicable), estimated completion date: December 2022	Ruty Shai, PhD Principal Investigator Sheba Medical Center	NCT04488783
Prospective pilot trial to assess a multimodal molecular targeted therapy in children, adolescent and young adults with relapsed or refractory high-grade pineoblastoma	Drug: Temozolomide Drug: Irinotecan Drug: Dasatinib Drug: Rapamycin (excipient palmitoyl ascorbic acid among others)	Oral administration, rapamycin (excipient palmitoyl ascorbic acid)	Up to 25 years (child, adult)	n=4 (e)	Recruiting (II), estimated completion date: April 2021	Selim Corbacioglu, MD Principal Investigator University of Regensburg	NCT02596828
Clinical trial of arsenic trioxide-combined chemotherapy in the treatment of stage 4 neuroblastoma	Drug: Arsenic trioxide Drug: Conventional induction chemotherapy	Intravenous infusion, 0.5-1.0 g of ascorbate/ infusion	Up to 14 years (child)	n=70 (e)	Recruiting (II), estimated completion date: December 2029	Yang Li Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University	NCT03503864
Medico-economic evaluation of surgery guided by fluorescence for the optimization of resection of glioblastomas	Drug: 5-Aminolevulinic acid (5-ALA) Drug: Placebo-ascorbic acid	Oral administration, 1 g of ascorbate before surgery	18 years and older (adult, older adult)	n=170 (a)	Passed its completion date (III), estimated completion date: August 2019, status has not been verified in more than two years, ascorbic acid well tolerated as control substance	Jacques Guyotat, MD Hospices Civils de Lyon	NCT01811121
Fluorescence-guided surgery for low- and high-grade gliomas	Drug: 5-Aminolevulinic acid (5-ALA) Drug: Placebo-ascorbic acid	Oral administration of 1.5 g of ascorbate before surgery	18 years and older (adult, older adult)	n=192 (e)	Passed its completion date (III), estimated completion date: June 2018, status has not been verified in more than two years, ascorbic acid well tolerated as control substance	Norissa Honea, RN, PhD St. Joseph's Hospital and Medical Center, Phoenix	NCT01502280

a, actual; e, estimated.

of 87.5 g consistently resulted in plasma ascorbate concentrations ≥20 mM (28,146). The absence of ascorbate-mediated serious adverse events in neither the radiation nor adjuvant phase enabled all subjects to maintain their performance status throughout treatment. Overall, pharmacological ascorbate was well tolerated and adverse events were restricted to dry mouth, fatigue, nausea, vomiting, infection, leukopenia, and neutropenia (rendering from grade 1-3, in total). The median overall survival was 18.3 months (146,147) compared to the historical median of 14 months according to Stupp *et al* (8). Therefore, it was found that subjects with unmethylated MGMT promoter as well as IDH wild type had longer overall and progression-free survival compared to other study participants. This treatment protocol for pharmacological ascorbate was applied for one woman with glioblastoma in New Zealand (147). She lived over four years from glioblastoma diagnosis largely experiencing good quality of life.

In the ongoing open-labeled, single group assigned Phase II trial NCT02344355 (active, not recruiting), intravenous infusions of 87.5 g of ascorbate are administered three times weekly during and after radiation. Additionally, ascorbate was planned to be administered twice weekly through the end of cycle six of temozolomide. The radiation and temozolomide treatments are based on the previously conducted phase I study NCT01752491 (8,28,146). According to this protocol, the overall and progression-free survival, the adverse event frequency as well as the quality of life were to be evaluated in a cohort of 90 glioblastoma patients. In extension of the data from Schoenfeld *et al* (28) and Allen *et al* (146), Cushing *et al* (148) established an application using magnetic resonance imaging in humans to visualize and measure changes in redox-active iron areas due to pharmacological ascorbate. In a small sample-sized study, 15 subjects from NCT02344355 underwent optical imaging procedures during application of high-dose ascorbate in addition to standard of care treatment. Five glioblastoma patients without ascorbate treatment were invited to participate as an active comparison group in the study. Although without statistical significance, due to the small number of subjects included in the study, the article described a method that can be employed to monitor *in vivo* changes in redox-active iron metabolism caused by the direct manipulation of endogenous redox state of iron in cancer subjects, which is in line with previous observations (28,134,148).

There are two more dose-escalation studies that investigated intravenous ascorbate treatments in patients with recurrent high-grade glioma (NCT02168270, NCT02833701), both performed at the University of Nebraska. In the Phase I study, no. NCT02833701, the role of bevacizumab and ascorbic acid was examined in nine patients with recurrent high-grade glioma. In the Phase I study, no. NCT02168270, temozolomide and ascorbic acid were tested in four participants. In both trials, patients received ascorbate intravenously over 90-120 min three times per week in addition to bevacizumab or temozolomide, respectively. Treatment was repeated every 28 days for up to 12 courses in the absence of disease progression or unacceptable toxicity for each study. The primary outcome measurements intended the determination of the maximum-tolerated dose of intravenous ascorbate given three times weekly in combination with temozolomide or bevacizumab and the evaluation of adverse events. Secondly, overall and progression-free survival, as well as quality of life were evaluated for treated patients. According to the available data sources, there are no dose-escalation protocols available for the two studies (NCT02168270 and NCT02833701). Phase I study NCT02168270 investigating the synergetic temozolomide and ascorbate therapy procedure was terminated in 2018 due to lack of efficacy and owing to non-completion of study protocol for all four participants. Serious adverse events occurred in two individuals; however, it was not specified if the serious adverse events were ascorbate- or temozolomide-induced. For Phase I study NCT02833701, which assessed the effects and best dose of ascorbate when given together with bevacizumab, no results are published. Although both clinical trials are already terminated there are no results about detailed study outcomes publicly accessible (NCT02168270 in 2018 and NCT02833701 in 2019).

Notably, in the randomized Phase 2 clinical trial NCT03503864, the therapeutic efficacy and toxicity of arsenic trioxide in the treatment of patients with recurrent or refractory stage 4 neuroblastoma was evaluated. This study is actually under recruitment and is being conducted at Sun Yat-Sen University in China. Owing to the study protocol patients are due to receive chemotherapy combined with conventional induction therapy, additionally for intravenous injection, arsenic trioxide and simultaneously 0.5-1.0 g ascorbate in 5% glucose solution. Treatment is scheduled every 28 days for a maximum of nine cycles in the absence of disease progression or unacceptable toxicity. Therefore, the focus has been on the 3-year overall survival rate, progression-free survival, as well as incidence of adverse events. The study outcomes from the experimental group, treated with arsenic trioxide combined with induction chemotherapy, should be compared with the results from the control group which only received conventional induction chemotherapy without arsenic trioxide.

In addition, there is a number of studies focusing on oral ascorbate in high-grade re-occurrent glioma, glioblastoma, low- and high-risk neuroblastoma or brain tumors (NCT01891747, NCT04488783, NCT01811121, and NCT01502280). Admittedly, the ingestion of orally administered ascorbate is tightly controlled, and intravenous administration enables bypassing of the control mechanisms and yields up to 100-fold higher plasma levels than those possible with maximal oral dosing. With intravenous administration, ascorbate is turned from vitamin to drug, acting as a pro-drug for H_2O_2 in the extracellular fluid, thereby having potential in the treatment of cancer (57,149). Therefore, the discussion of investigations with oral ascorbate supplementation is not in the scope of this review. The same applies for the two studies using palmitoyl ascorbate as excipient for rapamycin in the treatment of relapsed or refractory high-risk neuroblastoma and pineoblastoma in multimodal molecular-targeted therapy (NCT01467986 and NCT02596828). Overall, clinical trials employing intravenous vitamin C in critically ill patients (150-152) and patients with cancer (40,50,96,97,140) have demonstrated a lack of toxicity, good safety, and tolerability (141). In the current study Phase I (NCT01752491) (146) and one case report (147)

including glioblastoma patients, encouraging evidence was provided that receiving chemotherapy and radiotherapy together with high-dose ascorbate is beneficial.

7. Future directions and amplification of therapy effects

Modulation of microglia. Early studies on glioblastoma have been generally focused on the tumor-specific genetics and molecular profiles. However, tumor cells are not isolated and grow in a particular environment by communicating with other cell types, which influences progression, aggression, and survival of cancer. New investigations indicate that glioma cells interact in a complex way with their microenvironment promoting their proliferation, invasion, and treatment resistance (153).

Surrounding brain tumor-initiating cells are microglia, which are resident brain-specific immune cells of the CNS and distinct from peripheral circulating monocytes as well as other macrophages that have infiltrated from the circulation (154-158). These cells are believed to be initially recruited to eliminate the tumor by stimulating apoptosis of glioma cells (159) and by secreting inflammatory factors that prevent glioma growth and invasiveness (160). Under the influence of glioblastoma, microglia and macrophages are immunosuppressed and may contribute to glioblastoma invasion. Martins *et al* suggested that the local modulation of microglia within glioblastoma can control cancer cell progression by rendering microglial cell tumor-phagocytosis (161). Moreover, deficits of vitamin C in the brain have been observed in different neurological conditions and disorders, including Parkinson's and Alzheimer's disease, in which microglia pro-inflammatory activation influences their onset and/or progression (161).

Radiation and microglia homeostasis. Radiation therapy is one of the three standard of care components for glioblastoma. Radiation can kill proliferating tumor cells and severely impact the tumor microenvironment by altering the extracellular milieu at molecular and structural levels (162-164). Independent groups have documented enhanced human glioma cell migration and invasion in response to radiation (165-169). Gupta *et al* identified aggressive tumor behavior, microglial activation, and metabolic alterations with an increase of energy carriers (ATP and GTP) in the extracellular space as well as a decline in ascorbate in mice after radiation (170). Thus, microglia were observed to be amoeboid with higher phagocytic activity after radiation. In regard to the metabolic and morphologic changes in the radiated microenvironment, administration of ascorbate was suggested as an opportunity to decrease radiation-associated aggressiveness of recurrent glioblastoma and to enhance the long-term safety of brain radiation treatment for glioblastoma (170). SVCT2-deficient mice showed decreased ascorbate levels in the brain with a marked increase in cognitive deficits, amyloid β accumulation, and oxidative stress (171). Portugal *et al* showed that reduced vitamin C uptake through SVCT2 triggers the activation of primary rodent and human microglia. Thus, modulation of SVCT2 or vitamin C supply may be an attractive strategy for restoring microglia homeostasis and promoting neuronal viability (172). Moreover, mitochondrial SVCT2 was previously described in U937 cells (173) and HEK293 cells (174) and it was also observed in various cancer-derived cell lines from different origin (175). This observation was also correlated with cancer pathology (175) and absent in normal cells, suggesting that mitochondrial vitamin C may be relevant for cancer cell development or survival (176).

Modulation of BBB tightness. The BBB is a specialized non-permeable barrier in cerebral microvessels. Tight junctions between endothelial cells in brain capillaries are the most prominent characteristic of the BBB and are responsible for its selectivity (177). Higher supplementation of vitamin C in an *in vivo* mouse model revealed two times longer tight junctions than in low-dose supplemented animals (178). Behind the regeneration of DHA, derived from neuronal cells or delivered from blood, astrocytes also control BBB tightness. When these astrocytes become malignant as astrocytoma cells or are even turned into glioblastoma cells, the conditions within the brain become complex (179).

The impact of peroxiporins. Aquaporins (AQPs) have been described as crucial controllers of the BBB integrity and play an important role in cancer (180). Experimental evidence depicts that some AQPs also allow the transport of H_2O_2 through biological membranes. These AQPs are termed peroxiporins (181). Unlike normal cells, which are relatively unaffected by ascorbate, cancer cells exhibit a wide range of susceptibility depending on their catalase activity and plasma membrane permeability to H_2O_2. Erudaitius *et al* demonstrated that peroxiporin expression is an important factor for cancer cell susceptibility to therapeutic H_2O_2 (106). This suggests that cell susceptibility to ascorbate therapy is closely related to plasma membrane permeability to H_2O_2 in accordance with an elevated expression of peroxiporins. Therefore, modulation of plasma membrane is an option to increase the permeability of ascorbate. AQP1 and AQP4 upregulation which was observed in gliomas, glioblastomas, and also glioblastoma stem-like cells is believed to increase the perivascular space observed in gliomas (182-184). As H_2O_2 is known to be formed extracellularly as a byproduct of ascorbate oxidation and then permeating into the intracellular space through peroxiporin channeling (27,103), the efficacy of pharmacological ascorbate therapy (185) in glioblastoma may depend on the presence of H_2O_2 and on peroxiporins to pass through the plasma membrane, respectively.

Modulation of ascorbate transporter expression. It is possible that ascorbate delivery or uptake into the cell is also altered due to vascular dysfunction and disorganization (186). GLUT1 and to a lesser extent GLUT3 may be promising targets for glioblastoma treatment. The Warburg Effect occurring in cancer cells, is characterized by an extreme increase of glycolysis thereby decreasing the dependence on oxidative phosphorylation. Expression of GLUT1 and GLUT3 in glioblastoma tissue is often markedly altered (184,187,188). Co-culture experiments in the presence of ascorbate showed that tumor cells from CNS cells were able to acquire vitamin C via SVCT2; however, compared with normal cells, the capacity for ascorbate uptake was much lower in tumor cells (189). In this regard, it is important to emphasize

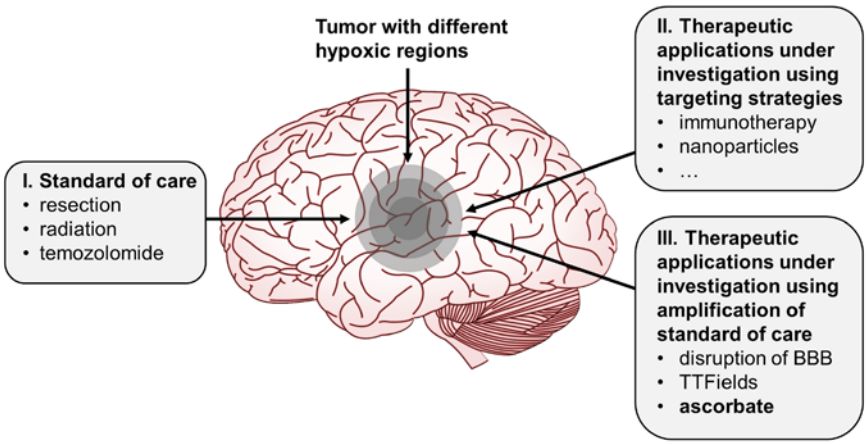

Figure 3. Overview of current therapeutic strategies and ongoing investigations in the glioblastoma treatment. There are three major parts in the treatment of glioblastoma: I. Standard of care (resection, radiation, and chemotherapy); II. Therapeutic applications under investigations using targeting strategies (immunotherapy, nanoparticles, and further therapeutic approaches); III. Therapeutic applications under investigation using amplification of standard of care [disruption of Blood Brain Barrier (BBB), TTFields, and ascorbate].

that the main form of vitamin C found after co-culture was ascorbate, meaning cancer cells are able to efficiently reduce DHA once inside the cell (175). Additionally, the competitive inhibition of GLUT transporters in glioblastoma cell lines did not suppress the ascorbate-induced toxicity suggesting that DHA is not the cancer cell-selective toxic species in this model (26,28,102,190). Reactive astrocytes generated following brain injury, neuroinflammation, stroke, aging, or neurodegenerative diseases, were able to induce SVCT2 expression, probably as a neuroprotective strategy for oxidative defense (191). On the other hand, reactive astrocytes release cytokines, chemokines, interleukins, nitric oxide, and other molecules, thereby promoting neuroinflammation (192) and the development of tumor microenvironment (155,189). Whether SVCT2 expression and function in reactive astrocytes may be considered a potential target for future clinical treatments remains to be further investigated.

The mechanism by which SVCT2 is upregulated in brain tissue is poorly understood. It has been observed that SVCT2 mRNA levels increase in neurons and astrocytes following oxidative damage due to ischemia and reperfusion (193). These findings underscore the importance of ascorbate as a neuroprotective agent and may have implications for therapeutic strategies. Induction of CNS inflammation resulted in the internalization and degradation of SVCT2 in microglia followed by the activation of NFκB (172). In C2C12 cells, it has been determined that the activation of NFκB and AP-1 would increase SVCT2 expression under conditions of oxidative stress generated by H_2O_2 (194). However, the whole picture of the interactions between microglia, astrocytes from tumor-unaffected regions, activated astrocytes, brain tumor-initiating cells, neurons, and tumor cells requires further exploration in glioblastoma patients and after treatment with high-dose ascorbate.

8. Conclusion

Despite advances in surgical techniques, radiation therapy, and chemotherapy, effective treatment of glioblastoma remains an unresolved challenge (8) (Fig. 3). In general, oncologic patients have decreased vitamin C levels, which are likely due to enhanced metabolic turnover as a result of oxidative and inflammatory aspects of the disease process (195). Nevertheless, the role of vitamin C in cancer patients is not fully clarified (73,196,197). Enhanced oxidative stress and pro-inflammatory biomarkers can also result from chemotherapy (198,199) or radiation (170). At present, only a small number of studies including glioblastoma patients and testing ascorbate as a potential therapeutic agent are available. The combination of pharmacological ascorbate with radiation and temozolomide provides hope to improve the patient's treatment performance as well as to ameliorate their life quality (47,146).

Although the data from *in vitro* and rodent models demonstrate effectiveness of ascorbate in the glioblastoma (146,147,200), its function mechanistically in healthy and glioblastoma subjects remains to be elucidated. Future investigations therefore have to clarify whether high-dose ascorbate is able to pass through the BBB in cancer patients, to reach all tumor cells at a sufficient concentration, to penetrate the hypoxic, inhomogeneous, and highly disorganized tissue, and to produce radicals as well as H_2O_2 to be pro-oxidative. Delivery of antioxidants such as vitamin C (or the more stable palmitoyl ascorbate) or α lipoic acid by liposomes seems to be a suitable approach to overcome limitations, including low bioavailability and instability, and can result in cancer tissue accumulation (201). These approaches to increase CNS drug levels are also promising strategies to apply high-dose ascorbate in glioma patients. Otherwise, the prolongation of supra-physiological ascorbate plasma levels over 2 h may act longer synergistically with drugs and positively increase the efficacy of therapy outcomes in general. On the other hand, making life more commodious for patients is imperative. Therefore, the reduction of ascorbate infusion's application frequency may contribute to a more satisfactory quality of life. Further development of nanomaterials-based strategies or other methods may contribute to improve ascorbate delivery to the tumor and to act on targeted transporters for inducing tumor-toxicities and cell protection, respectively. Sufficient

brain substance/drug delivery remains a major challenge for a wide range of neurological disorders and especially for glioblastoma. Nanomaterials can make use of mechanisms such as BBB-crossing mediated by cell-penetrating peptides (202), receptors (203), shuttle peptides (204), or even certain types of cells including macrophages, neutrophils, and monocytes (205). The combination of therapy regimes by integrating synergistic effects could overcome the limitations of a respective monotherapy and reinforce antitumor immunity or decrease tumor growth (206,207).

There is strong evidence that the effects of high-dose ascorbate treatment in glioma are not only restricted to general anti-oxidative effects that protect non-malignant and non-brain cells from the cytotoxic effects of chemotherapy and radiation (26,28,29,32). Additional investigations are required to explore whether adjuvant ascorbate therapy is also beneficial or even conflicting in combination with immunotherapy or oncolytic virotherapy treatment approaches.

Nevertheless, adjuvant high-dose ascorbate treatment in glioblastoma patients seems to be a promising therapeutic option that is able to improve the survival and quality of life of glioblastoma patients according to the available data from *in vitro* and *in vivo* studies. As the application of intravenous infusion is a routine standard procedure in the hospitalized patient's treatment, administration of pharmacological ascorbate requires no additional expertise for the staff, even more because the intravenous solutions are notably stable at ambient temperature (208).

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Availability and of data and materials

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Authors' contributions

OR, MB, TS, and SV wrote and conceived the manuscript. OR and MB carried out the literature and study research and generated the figures. HM and CV revised the manuscript. All authors read and approved the final manuscript. OR and MB confirm the authenticity of the data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

OR, MB, TS, and SV declare that they have no competing interests. CV and HM, as employees of Pascoe Pharmazeutische Praeparate GmbH (Giessen, Germany), received the ready designed/written manuscript for their review and were not involved in the content-related structuring. Furthermore, CV and HM had no influence on the content and interpretation of the literature used.

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