



## Utilization of redox modulating small molecules that selectively act as pro-oxidants in cancer cells to open a therapeutic window for improving cancer therapy

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### ABSTRACT

There is a rapidly growing body of literature supporting the notion that differential oxidative metabolism in cancer *versus* normal cells represents a metabolic frailty that can be exploited to open a therapeutic window into cancer therapy. These cancer cell-specific metabolic frailties may be amenable to manipulation with non-toxic small molecule redox active compounds traditionally thought to be antioxidants. In this review we describe the potential mechanisms and clinical applicability in cancer therapy of four small molecule redox active agents: melatonin, vitamin E, selenium, and vitamin C. Each has shown the potential to have pro-oxidant effects in cancer cells while retaining antioxidant activity in normal cells. This dichotomy can be exploited to improve responses to radiation and chemotherapy by opening a therapeutic window based on a testable biochemical rationale amenable to confirmation with biomarker studies during clinical trials. Thus, the unique pro-oxidant/antioxidant properties of melatonin, vitamin E, selenium, and vitamin C have the potential to act as effective adjuvants to traditional cancer therapies, thereby improving cancer patient outcomes.

### 1. Introduction

Metabolic alterations associated with neoplastic transformation and malignant progression are recognized as emergent hallmarks of cancer [1]. Recent theoretical constructs connecting cancer development to aging and malignant progression argue that oxidative metabolic abnormalities in dividing cells undergoing neoplastic transformation lead to acceleration of genomic instability and thereby drive malignant progression [2–4]. These models propose that genetic instability associated with carcinogenesis may be driven by perturbations in mitochondrial oxidative metabolism. Dysfunctional mitochondrial oxidative metabolism often leads to a “build-up” of electrons at sites capable of mediating one-electron reduction of O<sub>2</sub>, leading to an increase in the steady-state levels of intracellular reactive oxygen species (ROS) that are believed to contribute to initiation, promotion, and progression of the malignant phenotype.

Cancer cells are generally thought to have increased levels of ROS, *e.*

*g.* superoxide and hydrogen peroxide, compared to their normal cell counterparts [5–9]. Increased levels of ROS significantly contribute to: genomic instability, inability to perform differentiated function, immortalization, uncontrolled cellular proliferation, and the progression to the malignant state [2,3,5–7,10,11]. The altered metabolism of tumor cells was first reported by Otto Warburg in 1927 [12].

The Warburg effect describes the increased glucose uptake of tumor tissue relative to adjacent normal tissues. This increased glucose uptake may be utilized in the pentose phosphate pathway to generate reducing equivalents (*i.e.*, NADPH). Transketolase is a critical pentose phosphate pathway mediator necessary for regenerating NADPH. Recently, transketolase has been shown to be critical in regulating cancer cell redox metabolism in hepatocellular carcinoma cells [13]. In this model system, transketolase inhibition led to increased oxidative distress and enhanced sensitivity to sorafenib. By driving the detoxification of hydroperoxides *via* the glutathione peroxidases and peroxiredoxins, increased NADPH can help mitigate the consequences of increased levels of superoxide

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(O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in cancer cells under proliferating conditions [6,14]. Given the prooxidant-propelled metabolic alterations in cancer cells, relative to normal cells, we hypothesize that redox active small molecules that can selectively act as pro-oxidants in cancer cells may provide a novel, biochemical adjuvant to cancer therapy while simultaneously protecting normal tissues by functioning in an antioxidant capacity. Here we examine four redox active small molecules (i.e., melatonin, vitamin E forms, selenium, and vitamin C) that are well tolerated at pharmacological levels *in vivo* and have shown significant promise as adjuvants to cancer therapies (Table 1).

## 2. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) has been suggested to be a compound capable of preventing and treating several cancer types by suppressing pathways associated with the hallmarks of cancer, including the regulation of pro-survival signaling and tumor metabolism, the inhibition of angiogenesis and metastasis, and the induction of epigenetic alterations in oncogenes and tumor suppressors. Studies have also suggested that melatonin can reduce normal tissue injury associated with radiation and chemotherapy treatment, as well as enhance the efficacy of chemotherapy, thereby improving cancer survival rates [15].

Melatonin is a hormone secreted by the pineal gland known for its role in regulating circadian rhythms [16] and oxidative metabolism [17]. Melatonin secretion is regulated by the duration of light exposure; darkness is associated with high melatonin secretion. In addition to regulating circadian rhythms, melatonin exhibits anti-inflammatory,

**Table 1**

Clinical trials investigating traditional antioxidants as adjuvants to cancer therapy.

Cancer Type	Treatment	Phase	Trial Identifier <sup>a</sup>
Breast	Melatonin 3 mg	Early Phase 1	NCT01805089
Non-small Cell Lung	Dietary supplement: 20 mg melatonin vs. placebo	Phase 3	NCT00668707
Head and Neck	Dietary supplement: 40 mg melatonin vs. matched placebo	Phase 2	NCT02430298
Gastrointestinal and Lung	Dietary supplement: 20 mg melatonin vs. matched placebo	Phase 3	NCT00513357
Prostate	Dietary supplement: 400 IU vitamin E vs. placebo	Phase 3	NCT00809458
Head and Neck	Dietary supplement: 1000 mg vitamin E for 7 weeks	Phase 2	NCT02397486
Pancreatic	Dietary supplement: vitamin E $\delta$ -tocotrienol supplied as 100 mg, 200 mg, and 400 mg capsules	Phase 1	NCT00985777
Breast	Dietary supplement: tocotrienol 200 mg twice a day	Phase 2	NCT04496492
Soft Tissue Sarcoma	Intravenous high dose ascorbate (75 g) in combination with radiotherapy	Phase 1/2	NCT03508726
Pancreatic	Intravenous high dose ascorbate (75 g) in combination with radiation and chemotherapy	Phase 2	NCT02905578
Ovarian	Dietary Supplement: Oral ascorbate in combination with chemotherapy	Phase 2	NCT00228319
Bladder	Intravenous ascorbate in combination with chemotherapy	Phase 2	NCT04046094
Glioblastoma	Intravenous ascorbate (87.5 g) in combination with radiation and chemotherapy	Phase 2	NCT02344355
Renal Cell Carcinoma	Oral selenomethionine (4 mg) in combination with axitinib (5 mg)	Phase 1/2	NCT02535533

<sup>a</sup> ClinicalTrials.gov Identifier at <http://clinicaltrials.gov> as accessed 2020.11.05.

antioxidant, and oncostatic properties [16,17]. Once in circulation, melatonin is taken into cells via G-coupled receptors, melatonin receptors 1 and 2, which have picomolar and nanomolar binding affinities, respectively [18]. Intracellularly, melatonin accumulates in the mitochondria, where it can scavenge ROS [19] and regulate N-ribosylidihydroxynicotinamide (NRH):quinone oxidoreductase 2 (NQO2) and therefore modulate levels of ROS [17,20]. Melatonin is thought to chemically interact with various forms of ROS and reactive nitrogen species (RNS) to produce three main chemical products: N(1)-acetyl-N(2)-formyl-5-methoxykynurenin (AFMK), N(1)-acetyl-5-methoxykynurenin (AMK), and hydroxymelatonin (HO-melatonin) (Fig. 1) [21,22]. The generation of AFMK and HO-melatonin is dependent on the concentration of O<sub>2</sub>. AFMK is the primary product in aerated solutions (84%), while HO-melatonin is prevalent in low O<sub>2</sub> environments (86%). Because human tissues are typically at 4–5% O<sub>2</sub>, HO-melatonin is the most physiologically relevant intermediate of melatonin [23]. For this reason, 3OH-melatonin is considered a potential biomarker of *in vivo* ROS levels [24]. It is thought that AMK, AMFK, and cyclic 3HO-melatonin are better HO<sup>•</sup> and HO<sub>2</sub><sup>•</sup> scavengers than melatonin [25,26]. In addition to its ability to act as a free radical scavenger, melatonin may also chemically react with redox active metals contained within the cell to blunt the catalysis of oxidation reactions (e.g. Fenton reactions of iron). Melatonin has been shown to mitigate the activity of the heme containing protein, myeloperoxidase [27]. Melatonin has also been shown to mitigate both estradiol-induced, oxidative DNA damage (e.g. 8-oxoguanine) and iron-catalyzed lipid peroxidation [28,29]. Along with iron, there is also evidence of melatonin being able to decrease copper-mediated DNA damage *in vivo* [30]. Thus, it may be well reasoned to consider melatonin as a potential metal chelator. Due to its apparent antioxidant capacity and potential to interact with catalytic metals, melatonin is currently being investigated as a supplement for both cancer prevention and treatment.

### 2.1. Anti-cancer effects of melatonin

Increased blood melatonin levels initiate homeostatic metabolic rhythm signals to enter a state of rest. Light exposure at adverse times can disrupt circadian rhythms and may contribute to the development, promotion, and progression of cancer [31]. Artificial light exposure during normal circadian rest cycles is associated with reduced melatonin secretion and an increased risk of breast cancer [32]. Reduced secretion of melatonin may lead to an increase in reproductive hormone, including estradiol. Increased levels of estradiol are associated with an elevated risk of breast cancer development [33]. In support of this, *in vitro* experiments performed in estrogen receptor-positive MCF-7 human breast cancer cells demonstrated that melatonin, at a physiological concentration (1 nM) and in the presence of estradiol, inhibits cell proliferation, increases the expression of p53 and p21/WAF1 proteins, and counteracts the stimulatory effect of estradiol on cell invasiveness. Melatonin's effect on invasion and metastasis is partly mediated by a melatonin-induced increased expression of the cell surface adhesion proteins E-cadherin and beta(1)-integrin [34]. Physiological concentrations of melatonin have been shown to decrease the growth rate of breast cancer cells by increased expression of p21/WAF1 along with p53 [34]. Increased expression of p53 promotes genetic stability and makes cells less prone to tumor formation [35]. Melatonin has also been shown to be pro-apoptotic in colorectal cancer cells when given in combination with a somatostatin analog [36].

Melatonin has been shown to enhance the expression of the antioxidant enzymes catalase, superoxide dismutase, and glutathione reductase [37,38]. Activation of this antioxidant network has been shown to reduce intracellular ROS levels and promote cellular differentiation in prostate cancer cells [5,10,39,40]. While many researchers attribute the anticancer effects of melatonin to its antioxidant function, there is some suggestion that it may function as a pro-oxidant in cancer cells [41,42] by selectively disrupting mitochondrial and metal ion metabolism. In

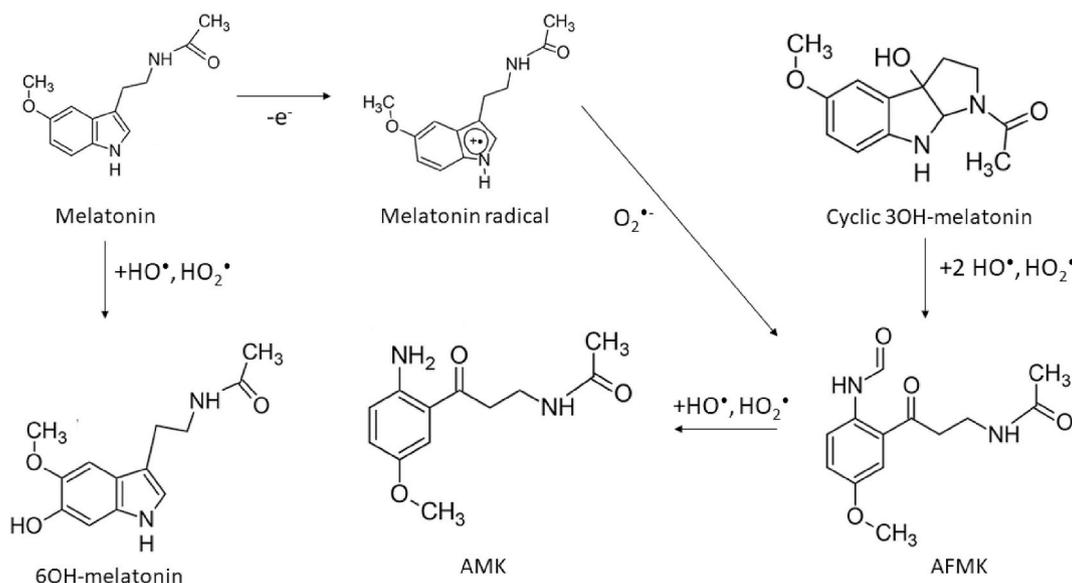


Fig. 1. Schematic of melatonin and associated metabolites reactions with reactive oxygen species [22].

human leukemia Jurkat cells, high concentrations of melatonin (10–1000  $\mu\text{M}$ ) led to Fas-induced apoptosis *via* ROS generation [43]. Rodogna et al. showed that 1 mM melatonin induced ROS production as early as 1 min following exposure with ROS and persisting up to 6 h post-treatment [44]. High-doses (1–10 mM) of melatonin elevate ROS within 15 min in HepG2 cells [45]. The pro-oxidant nature of high-dose melatonin has been shown to promote apoptosis *via* caspase activation and can be counteracted by N-acetyl-L-cysteine, Trolox, PEG-catalase, and glutathione [46]. The fundamental pro-oxidant mechanism of high-dose melatonin is still poorly understood. MT1 and MT2 receptors are not believed to be central to the pro-oxidant role of melatonin, because MT1/2 antagonists could not mitigate melatonin-mediated ROS production in U937 cells [44].

While the role that MT1/2 plays in carcinogenesis is unclear, it appears there may be differential expression amongst tumors. The melatonin receptor, MT2, has recently been shown to have differential expression among lung cancer subtypes, with higher MT2 expression being associated with a more favorable prognosis [47]. This suggests the potential utility of melatonin agonists, such as ramelteon or agomelatine, to function as anti-neoplastic agents. Both ramelteon and agomelatine are promising because they have greater bioavailability than melatonin itself [48]. The plasma half-life of agomelatine is  $\approx 2$  h [49] and  $\approx 1$ –2 h for ramelteon [50,51]. While primarily for use as treatments for insomnia and depression, ramelteon and agomelatine have garnered interest as cancer therapeutics [48]. Despite their theoretical clinical promise, the anti-cancer potential of both ramelteon and agomelatine need to be investigated.

The pro-oxidant nature of melatonin may be partly due to disruptions in the electron transport chain (ETC), a hypothesis based on the preferential accumulation of melatonin in the mitochondria [19,52]. In human mesangial cells and mouse kidney mitochondria, high doses of melatonin disrupt complex III of the ETC, leading to increased superoxide formation [53,54]. High-dose melatonin has also been shown to increase nNOS expression transiently *in vitro*, resulting in a decrease in oxidative phosphorylation and mitochondrial membrane potential [55, 56]. The definitive role of the ETC disruptions by melatonin has yet to be elucidated but shows great promise since it is well known that cancer cells have significantly elevated levels of ROS relative to normal cells [3, 5,40].

## 2.2. Normal tissue protection

In addition to its potential for enhancing cancer therapy, melatonin may also protect normal tissues from cancer therapy-associated toxicity by assuming a role as a donor antioxidant at higher doses, inducing NQO2, which is able to reduce quinones and semiquinones to hydroquinones, and/or acting as a chelator of redox active metal ions [17,20, 42]. The chemotherapy agent, adriamycin, is associated with significant heart and liver toxicity *via* oxidative damage. In 2008, a study of adult male rats given a single dose of 10 mg kg<sup>-1</sup> adriamycin had significant increases in creatine kinase, lactic dehydrogenase, and aminotransferases, circulating iron, ferritin, and transferrin [57]. These elevations correlated with a decrease in liver and heart glutathione and glutathione-S-transferase activity, along with increases in lipid peroxidation, protein oxidation, catalase activity, and glutathione peroxidase activity. The supplementation of melatonin to adriamycin-therapy returned hepatic and cardiac function markers, circulating iron markers, and TBARS and protein carbonyl levels to baseline levels. Glutathione and glutathione peroxidase activity in the heart and liver also returned to baseline levels following the addition of melatonin to Adriamycin. This study presents an example of the potential novelty of melatonin supplementation during cancer therapy to mitigate normal tissue injuries.

Melatonin has also demonstrated significant activity as a radioprotector [58], protecting against radiation-induced DNA damage [59]. More recently, the combination of melatonin and vitamin C reduced DNA damage in peripheral blood samples [60]. In this study, 15 healthy volunteers were given an oral dose of 300 mg melatonin and 300 mg vitamin C with peripheral blood being collected 1 h, 2 h, and 3 h following supplementation. The blood was irradiated with 200 cGy of 6 MV photons and assessed for nuclear fragmentation. Maximum protection occurred at 1 h following oral supplementation. In addition, melatonin recently has been shown to mitigate radiation-induced lung fibrosis in a preclinical animal model [61]. Male C57BL/6 mice were treated with 1 mg melatonin daily for 7 days following a single dose of 15 Gy to the lungs. Mice that received melatonin had significantly reduced oxidative stress markers and macrophage infiltration in the lungs following radiation.

Further support for the hypothesis that melatonin may mitigate cancer therapy-associated toxicity arises from its ability to protect brain tissue from neurotoxins [62]. Melatonin protects the brain from oxidative injuries from methamphetamine [63–66], neural lipid peroxidation

associated with aminolevulinic acid accumulation [67,68], and mitochondrially produced  $O_2^{\cdot-}$  from rotenone [69]. The extensive literature on melatonin's ability to protect CNS structures from a wide variety of neurotoxins suggests that melatonin may also protect against neurotoxicity following cancer therapy.

### 2.3. Clinical relevance

The ideal dosage for melatonin as a potential cancer preventative or cancer therapy adjuvant has yet to be determined. Oral doses of melatonin ranging from 20 to 40 mg given daily, gradually throughout the day, have been well tolerated [70–72]. Di Bella has reported that supraphysiological doses delivered intravenously up to a maximum dose of 1 g have limited side effects [73]. The primary side effect reported with 1 g given intravenously was drowsiness, usually at the beginning of the treatment [16]. A recent review of the clinical pharmacokinetics of melatonin documented the time to maximum serum concentrations being approximately 50 min [74]. Both the oral and intravenous half-life of melatonin is approximately 45 min (28–126 min) [74]. Oral bioavailability of melatonin is low with significant individual variability (9–33%) [74]. A separate review recognized that intranasal melatonin has substantially higher bioavailability of approximately 55–94% [75].

There are a limited number of completed clinical studies investigating melatonin's utility as an adjuvant to cancer therapy (Table 2). The Di Bella Method (DBM) [73] suggests that melatonin is clinically useful for the enhancement of cancer therapy while mitigating the high levels of toxicity. Note that this method also includes various other antioxidants and immunomodulators such as vitamin E, C, and D3, interleukin 2, and retinoids [16,76,77]. Retrospective clinical studies using the DBM have shown its potential to improve the therapeutic outcomes of patients with breast and head and neck cancer [60–62,68]. In 2005, Mills et al. published a meta-analysis of all randomized, controlled clinical trials utilizing melatonin in cancer patients with solid tumors [78]. By surveying electronic databases, they found 10 randomized, controlled clinical trials utilizing melatonin from 1992 to 2003 including a total of 643 patients. From each of these studies, the authors concluded that melatonin reduced the risk of death at 1 year (relative risk = 0.66, 95% confidence interval: 0.59–0.73) with no severe adverse events.

A more recent meta-analysis of melatonin in combination with chemotherapy, radiotherapy, or both was published in 2012 by Wang et al. [79]. This study evaluated 8 randomized, controlled clinical trials from 1992 to 2011 using an oral dosage of 20 mg daily in patients with solid tumors. They found that melatonin significantly increased both complete and partial remission (16.5% and 32.6%, respectively), increased the 1-year survival rate from 28.4% to 52.2% with a relative risk of 1.9 (95% confidence interval: 1.28–2.83), and decreased radiotherapy-related side effects including thrombocytopenia (19.7% vs. 2.2%, relative risk = 0.13), neurotoxicity (15.2% vs. 2.5%, relative risk = 0.19), and fatigue (49.1% vs. 17.2%, relative risk = 0.37). All reported effects were independent of cancer type and no severe adverse events associated with melatonin were reported. Despite the great promise for

the use of melatonin as an adjuvant to cancer therapy, further investigation into the utility of melatonin, both mechanistically and translationally, is warranted. In prospective clinical trials, oral melatonin has been shown to enhance the effectiveness of traditional chemotherapy approaches, such as cisplatin and etoposide [80], tamoxifen [81], and irinotecan [82].

In addition to its potential ability to enhance cancer therapy, supplementation with melatonin during treatment may improve patient quality of life. A double-blind, placebo-controlled, randomized clinical trial showed that patients receiving 6 mg oral melatonin daily had a lower risk of developing depressive symptoms [84]. This trial also showed that melatonin supplementation significantly improved sleep efficiency by reducing waking episodes after sleep onset [85]. A separate randomized, placebo-controlled trial reported significant increases in subjective sleep quality [86]. Another placebo-controlled crossover trial suggested that 20 mg melatonin daily improved fatigue in patients with advanced cancer [87]. These data suggest that melatonin has potential to mitigate negative side effects often experienced by cancer patients throughout the course of treatment and further enhances the notion that melatonin may mitigate normal tissue injuries as well.

### 3. Vitamin E

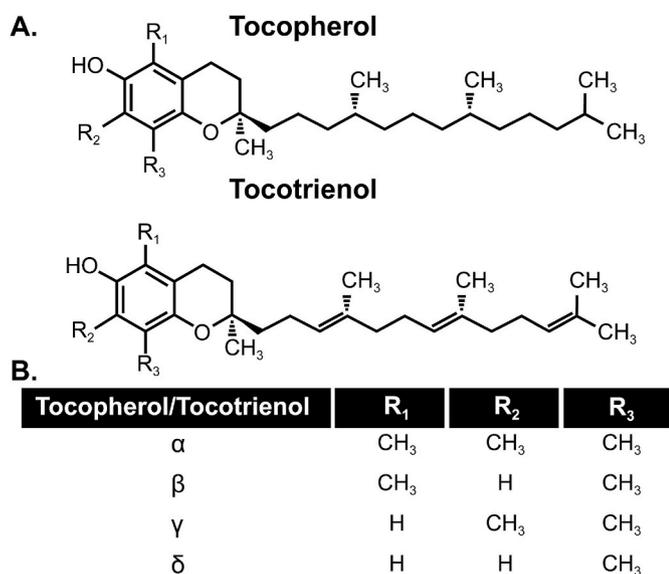
Nuts, plant seeds, and plant oils are rich in vitamin E and selenium [88]. There are several natural forms of vitamin E, namely,  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherol (TOH) and tocotrienol (TE-OH) [89] and have been suggested to have both antioxidant effects in normal tissues as well as pro-oxidant effects in cancer cells [90]. Structurally, tocopherol and tocotrienols isoforms are similar but tocopherols have a saturated side chain while tocotrienols have 3 double bonds on the side chain (Fig. 2). The combination of phenolic group on the chromanol ring and the phytol (or phytol-like) tail allows all vitamin E isoforms to be highly lipophilic antioxidants that can readily donate a hydrogen atom to scavenge lipid peroxide radicals in lipid regions of membranes as well as low density lipoprotein [91].

Lipid peroxidation chain reactions can be initiated by 1-electron oxidants (Fig. 3). This results in the formation of carbon-centered lipid radicals (PL $^{\cdot}$ ), which upon reaction with oxygen to form a phospholipid peroxy radicals (PLOO $^{\cdot}$ ) [92–94]. Peroxy radicals can then initiate new chains of free radical-mediated oxidations resulting in the formation of phospholipid hydroperoxides (PLOOH). However, there is a more biochemical energetically favorable way to produce the PLOOH [94]. The rate constant of inter-lipid propagation reactions is relatively low ( $k \approx 10 \text{ M}^{-1} \text{ s}^{-1}$ ) [95]. Vitamin E can outcompete the propagation reaction with a much higher rate constant ( $k \approx 8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ) [96] to also form PLOOH while preventing further propagation. The residual vitamin E radical (TO $^{\cdot}$ ) is then recycled by vitamin C via a water-lipid interface interaction [94,97,98]. The vitamin C (Asc $^{\cdot+}$ ) radical dismutates, forming AscH $^-$  and dehydroascorbate [94]. DHA is relatively harmless due to its rapid reduction back to AscH $^-$ .

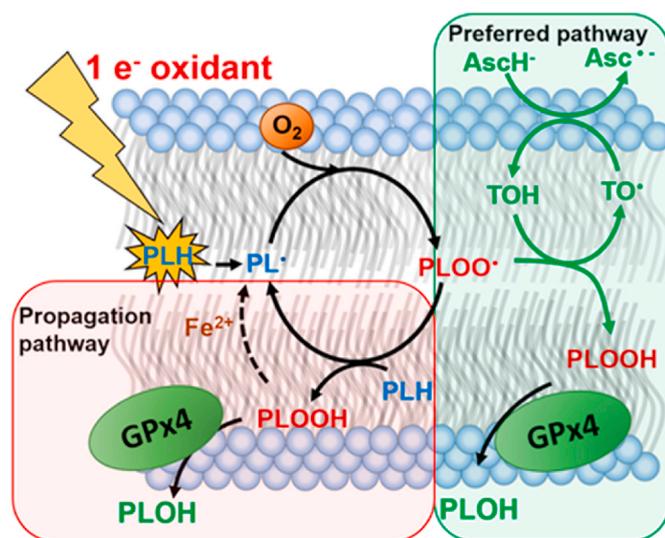
Collaboratively with vitamins E and C, the metalloid selenium (Se), when incorporated in glutathione peroxidase-4 (GPx4; associated with

**Table 2**  
Recently completed clinical trials utilizing melatonin as an adjuvant therapy.

Cancer Type	Dosage	Combination of Drugs	Results	References
Metastatic Colorectal Cancer	melatonin (40 mg/day orally)	low-dose subcutaneous interleukin-2	Significantly increased 1-year survival rate of patients	[83]
Metastatic Breast Cancer	melatonin (20 mg/day) orally starting 7 days before tamoxifen	Tamoxifen	Partial response in 4/14 (28.5%) patients, improved anxiety in most patients and did not enhance the toxicity of tamoxifen. Serum levels of IGF-1 were decreased by the combination therapy.	[81]
Metastatic NSCL Cancer	melatonin (20 mg/day) orally in the evening	cisplatin and etoposide	Improved overall tumor response rate and 5-year survival, with better tolerance to chemotherapy	[80]
Breast Cancer	Di Bella Method	somatostatin, retinoids, vitamin D $_3$ , and low dose of cyclophosphamide	Positively correlated with survival and tumor response	[77]



**Fig. 2.** Chemical structure of Vitamin E. A. Structural comparison of tocopherol and tocotrienol forms. B. Side chain moieties of individual tocopherols and tocotrienols.



**Fig. 3.** The antioxidant triad, vitamins C, E and selenium cooperatively terminate lipid peroxidation. Oxidation of phospholipids can be initiated by 1-electron oxidants. Upon oxygenation, a phospholipid peroxy radical, PLOO<sup>•</sup>, is formed. This species can be protonated through two pathways to form PLOOH. The first pathway, in green, utilizes vitamin C and E as antioxidants thus, protecting adjacent phospholipids to form PLOOH. The second pathway abstracts a hydrogen atom of another phospholipid to form PLOOH. However, this creates a new lipid radical PL<sup>•</sup>, and thus propagates lipid peroxidation. As shown, both pathways create PLOOH, but the antioxidant pathway is preferred, because this does not reinitiate lipid peroxidation reactions. The formed PLOOH is a substrate for GPx4 that detoxifies it to an alcohol, terminating lipid peroxidation. If GPx4 activity is blunted, PLOOH can initiate chain-branching lipid peroxidation reactions when ferrous iron is available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the lipid bilayers) and glutathione peroxidase-1 (GPx1; in the mitochondrial matrix and cytosol) assist the termination of lipid peroxidation [99]. GPx4 detoxifies PLOOH and converts it to its corresponding alcohol while GPx1 reacts with both H<sub>2</sub>O<sub>2</sub> and ROOH to form either H<sub>2</sub>O or ROH. If the hydrogen atom transfer of vitamin E to a lipid peroxy

radical is too slow, perhaps due to low levels of TOH, PLOO<sup>•</sup> will abstract a hydrogen atom from a neighboring phospholipid, thereby propagating the chain reactions of lipid peroxidation. In addition, insufficient GPx4 activity can increase the steady-state level of PLOOH, providing the potential for additional free radical oxidations.

Low GPx4 activity can be caused by selenium deficiency, which can be overcome by supplementation of Se [99]. During Se-deficiency or inhibition of GPx4, the higher steady-state level of PLOOH could lead to more reactions of ferrous iron through a Fenton-like reaction with PLOOH, leading to PLO<sup>•</sup>, which initiates new chain-branching free radical reactions [100–102]. Therefore, the synergy of vitamin E and selenium are thought to be novel partners that can prevent these free radical-mediated oxidations that could lead to cancer or be used in the treatment of cancer.

While GPx4 appears to play a major role in the Se-dependent antioxidant effect of lipid peroxidation, other Se-dependent enzymes should not be overlooked. A different isoform in the GPx family, GPx1, also has Se at its active site. GPx1 reduces hydrogen peroxide as well as organic hydroperoxides in the cytosolic fraction of cells. Cytosolic hydroperoxides consist of lipid sections that were cleaved of oxidized phospholipids by phospholipase A<sub>2</sub> [103]. Like GPx1, the 2-Cys members of the peroxiredoxin family (prxd1-5) can also act on these organic hydroperoxides [103,104]. In contrast to GPx4 termination of oxidation within the lipid bilayer, both GPx1 and the 2-Cys peroxiredoxins detoxify downstream products of lipid oxidation. The peroxiredoxins overlap with the glutaredoxins in the antioxidant recycling system, as described by Ng et al. [105]. Both, the peroxiredoxins and the glutaredoxins receive electrons from the pentose phosphate pathway, and thus NADPH. The flow of electrons occurs via the reductase/glutaredoxin pathway (GR/GRX), and the Se-dependent thioredoxin/thioredoxin reductase (Trx/TrxR) pathway [106–109]. This shows how Se-dependent enzymes, besides GPx4, can contribute to the termination of lipid peroxidation.

### 3.1. Cancer prevention

Vitamin E has been well investigated as an antioxidant supplement for cancer prevention. In 1994, a randomized, double-blind, placebo-controlled trial examined vitamin E's ability to reduce the incidence of lung cancer in male smokers (the study also tested beta-carotene and a combination of vitamin E and beta-carotene, 87). The trial found no difference in the incidence of lung cancer [110]. Similarly, a study of Finnish male smokers who consumed vitamin E supplements for five to eight years failed to show a significant decrease in the incidence of pancreatic carcinoma [111]. This same cohort was also analyzed for the incidence of urinary tract cancer (urothelial cancer and renal cell cancer) and again showed no beneficial effect of vitamin E on cancer initiation [112]. In contrast, the same Finnish male smokers did show a modest preventative effect of colorectal cancer risk with long-term supplementation of vitamin E [112]. Likewise, a study conducted between 1992 and 2004 that recruited healthy US women and randomly assigned them to receive vitamin E or placebo reported no overall benefit for cancer prevention [113]. The SELECT trial was a randomized, placebo-controlled trial of vitamin E supplementation for 7–12 years ability to prevent prostate cancer in healthy men (the study also investigated selenium supplementation, or a combination of vitamin E and selenium, 91). This trial failed to show a benefit in prostate cancer incidence [114]. Recent reviews have suggested that supplementation of vitamin E and selenium may only be effective in cancer prevention when the subjects are deficient but not when are sufficient in these nutrients [115,116]. Based on these results, the hope of using supplemental vitamin E as a means of cancer prevention has waned.

### 3.2. Anticancer mechanisms of vitamin E

The biochemical anticancer pro-oxidant effects of vitamin E have

been extensively studied and are thought to be mediated by tocopheroxyl/tocotrienol-radical formation from mitochondrial metabolism in cancer cells [89,90,117–119]. The enhanced efficacy of  $\gamma$ -TE-OH may be partly explained by its increased accumulation in cancer cells compared to the tocopherols [120]. Tocotrienols have also been shown to accumulate selectively in cancer cells relative to normal cells following oral administration [96,97]. The tocotrienols  $\gamma$ -TOH,  $\delta$ -TOH,  $\gamma$ -TE-OH, and  $\delta$ -TE-OH have all been shown to be effective in inducing growth arrest, apoptosis, autophagy, and endoplasmic reticulum stress in cancer cells [121–125]. The tocotrienols  $\gamma$ -TE-OH and  $\delta$ -TE-OH, specifically, are believed to be much stronger anticancer agents than the tocopherols, with an IC<sub>50</sub> range of 10–20  $\mu$ M, as compared to  $\geq 25$ –50  $\mu$ M [95,99,100] — all of which are thought to be more potent anticancer agents than  $\alpha$ -TOH. By direct comparison, 50  $\mu$ M  $\alpha$ -TOH failed to produce any reduction in viable PC-3 cells, while an equivalent dose of  $\gamma$ -TOH reduced cell viability by approximately 50% [126]. As for  $\gamma$ -TE-OH, it has been shown to be capable of slowing cell growth and activating apoptosis in both prostate and breast cancer cells [95,100,102,103]. This is thought to be related to the mitochondrial electron transport chain II targeting of redox metabolic differences between cancer versus normal cells [129,130].

In both prostate and breast tumor cells,  $\gamma$ -TE-OH induces apoptosis *via* caspase activation by suppressing Id1 and NF- $\kappa$ B *via* activation of the proapoptotic JNK pathway [95,102]. Other antiproliferative and cell stress-associated pathways affected by  $\gamma$ -TOH and  $\gamma$ -TE-OH include PPAR- $\gamma$  upregulation [127,128,131], inhibition of P13K-mediated AKT phosphorylation [95,102,105], increased caspase 8/9 activation, and expression of the autophagy marker LC3II [96,102,106]. A possible biochemical mechanism for the anticancer effects of vitamin E forms may be the modulation of sphingolipids. Persistent accumulation of sphingolipids in cancer cells is known to induce apoptosis and inhibit cell growth [132–134]. Both  $\gamma$ -TOH and  $\gamma$ -TE-OH elevate dihydrosphingosine, dihydroceramides, and ceramides in prostate [126], breast [135], colon [136], and pancreatic [137] cancers. One mechanism of sphingolipid accumulation occurs through dihydroceramide desaturase inhibition during *de novo* synthesis of sphingolipids, potentially leading to enhanced sphingomyelin hydrolysis [137]. Blocking *de novo* synthesis of sphingolipids can reverse the anticancer effects of both  $\gamma$ -TOH and  $\gamma$ -TE-OH [126,135,137]. Inhibition of sphingolipid synthesis prohibits  $\gamma$ -TOH and  $\gamma$ -TE-OH apoptotic induction *via* endoplasmic reticulum stress-induced activation of the JNK/CHOP/DR5 pathway [135]. Taken together these data suggest that vitamin E isoforms interact with sphingolipids to enhance cellular stress and promote cancer cell death.

### 3.3. Vitamin E as a potential adjuvant therapy

While the various forms of vitamin E are structurally similar, they greatly vary in bioavailability. Of the eight isoforms of vitamin E, the most readily available in plasma and tissues is  $\alpha$ -tocopherol [138]. Serum concentrations of  $\alpha$ -tocopherol range from 20 to 50  $\mu$ M and have a half-life of approximately 30 h but is dependent on the  $\alpha$ -tocopherol transfer protein expression [139,140]. The other vitamin E isoforms have a 4–8 times shorter half-life and much lower serum concentrations [139]. With supplementation,  $\gamma$ -tocopherol has a serum concentration of 10–30  $\mu$ M [141,142]. An oral dose of 300 mg  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocotrienol has been shown to have a plasma half-life of 4.4, 4.3, and 2.3 h, respectively [143]. Although most cancer prevention trials utilizing vitamin E were unsuccessful, there is growing interest in the utility of vitamin E forms as adjuvants to cancer therapy (Table 3). In a phase I pharmacokinetic study,  $\delta$ -TE-OH was given to patients in an oral dose range of 200–1600 mg daily [144]. Serum concentrations up to 18  $\mu$ M  $\delta$ -TE-OH were achieved and were sufficient to activate caspase-3 in dysplastic and malignant tissues [144]. At this dose level, no dose-limiting toxicities were observed [144]. There is concern of increased mortality associated with high dose (>400 mg) vitamin E due to cardiovascular complications

**Table 3**

Recently completed clinical trials utilizing vitamin E forms as adjuvant therapy.

Cancer Type	Dosage	Results	References
Pancreatic Cancer	$\delta$ TE at escalation doses of 200–3200 mg/d for 2 weeks before surgery	$\delta$ TE is generally safe and induced apoptosis in dysplastic or malignant tissues from pancreas	[144]
Colorectal Cancer	$\gamma$ TmTs for 1 or 2 weeks	Bioavailability, plasma P2-isoprostanate, inflammation markers	NCT00905918
Ovarian Cancer	Cabazitaxel (25 mg/m <sup>2</sup> ) vs. tocotrienol (300 mg $\times$ 3); 3 months	Survival rate and cancer progression	NCT02560337
Lung Cancer	Tocotrienol, 300 mg $\times$ 3 plus standard chemotherapy	Disease progression-free survival	NCT02644252

[145]. A meta-analysis from 2012 of 19 independent clinical trials found there was a significant increase in the all-cause mortality rate (39 per 10,000 persons) for high dose vitamin E (>400 mg) compared to low dose supplementation (16 per 10,000 persons) [146]. The increased mortality associated with high dose vitamin E may be primarily as a result of cardiometabolic effects. In 2005, a randomized, double-blind, placebo-controlled trial evaluating the effects of high dose vitamin E supplementation (400 mg daily) on patients at risk for cardiovascular disease was completed [147]. The long-term study followed patients (n = 3994) for a median of 7 years. The results of this trial showed that patients that received a 400 mg vitamin E supplement were at risk for cardiovascular disease (relative risk = 1.13; 95% CI, 1.01–1.26; P = .03) and hospitalization associated with cardiovascular disease (relative risk = 1.21; 95% CI, 1.00–1.47; P = .045). Despite showing minimal toxicities in cancer related trials, there may be significant long-term effects associated with chronic, high dose vitamin E supplementation that warrant consideration.

One form of vitamin E often considered as a cancer therapy is the tocotrienol-rich fraction (TRF) extracted from palm oil [89]. TRFs have been shown to have an antitumor effect in breast cancer [148] and lung cancer [149] in preclinical animal models. A double-blind, placebo-controlled trial utilizing oral TRFs (200 mg or placebo control) in combination with tamoxifen (20 mg daily) in stage I/II estrogen receptor positive breast cancer found a 5-year disease-free survival rate of 86.7% compared to 83.3% in the control group [150]. The risk of mortality due to breast cancer was found to be significantly reduced by TRF supplementation (hazard ratio = 0.4, 95% confidence interval = 0.08–2.05) [150].

In preclinical studies, tocotrienols have shown promise as a potential adjuvant to radiation therapy. Kumar et al. showed that when nude mice with PC3 tumor xenografts were given 400 mg kg<sup>-1</sup>  $\gamma$ -TE-OH 24 h before a single dose of 12 Gy radiation, there was a 40% reduction in tumor volume compared to reduction by radiation alone [151]. The addition of  $\gamma$ -TE-OH was shown to have no toxic effects in the rectum and was slightly protective of the liver [151]. Unfortunately, the authors did find that the addition of  $\gamma$ -TE-OH appeared to sensitize the kidneys to lipid peroxidation along with the tumor [151].

### 3.4. Normal tissue protection by vitamin E

Tocotrienols may also protect normal tissues from radiation-induced damage. Mice receiving a single dose of 200 mg kg<sup>-1</sup>  $\gamma$ -TE-OH before 8 Gy total body irradiation (TBI) exposure were able to protect hematopoietic stem cells and progenitor cells compared to mice receiving radiation alone [152]. A single dose  $\gamma$ -TE-OH (400 mg kg<sup>-1</sup> given subcutaneously 24 h before 9 Gy TBI) was able to improve post-radiation survival, decreased radiation-induced vascular oxidative stress, and protected mice from gastrointestinal injury [153]. Similar to  $\gamma$ -TE-OH, a

single dose of  $\delta$ -TE-OH (400 mg kg<sup>-1</sup> given subcutaneously before radiation) protected 100% of mice from TBI-induced death measured 30 days after radiation [154]. Subcutaneous  $\delta$ -TE (75–100 mg kg<sup>-1</sup>) given to mice before 12 Gy TBI protected the animals from TBI-induced gastrointestinal damage, as evidenced by an increased number of jejunal crypt cells present 30 days post-radiation relative to radiation alone [154].

#### 4. Selenium as a cancer therapeutic

Redox-active inorganic Se-compounds, such as sodium selenite, are known to be pro-oxidant at high doses [155–157]. The associated increase in formation of ROS may tip the redox status of cancer cells and thus, killing the cells. Cells in healthy tissues can cope with these fluxes of oxidative stress, while cancer cells are at the limit of their ability to control the oxidative distress, thus killing them. A variety of oxidative damages of redox-active selenium compounds have been reported: sodium selenite was shown to induce single-strand DNA breaks [158–162], as well as oxidative DNA lesions e.g. 8-hydroxydeoxyguanosine [163]. As a result, apoptosis can be readily observed in cells treated with single digit  $\mu$ M concentrations of sodium selenite. Additionally, a different study showed inhibition of neoplastic growth of HeLa cells upon treatment with selenite (5  $\mu$ M). In contrast, Se-DL-cystine, a non-redox active selenium compound needed a much higher concentration (100  $\mu$ M) to show a similar anti-tumor response [164]. This last effect is an appropriate example of how understanding and selecting the right Se-compound for each study important. Generally, organic selenium compounds, e.g. selenomethionine, methyl-selenocysteine or Se-DL-cystine are less toxic at higher concentrations. Because these compounds are not redox active, they do not generate ROS readily. An extensive overview on selenium compounds for cancer treatment are provided in Ref. [165].

Zakharia et al. employ a different strategy to utilize selenium in cancer therapy. Where inorganic sources are typically used to induce oxidative distress, this clinical trial (NCT02535533) uses high dose seleno-L-methionine (SLM), a well tolerated, organic source of Se [166, 167]. Preclinical work suggests that high-dose SLM results in down-regulation of hypoxia induced factor 1 $\alpha$  and 2 $\alpha$  (HIFs) and downstream vascular endothelial growth factor (VEGF) and associated oncogenic miRNA 155 and 210, which results in reduced vascular permeability and improved drug delivery into the tumor upon treatment with high-dose SLM [168–175]. These preclinical data provided the rationale for the use of a defined dose and schedule of SLM in sequential combination with standard of care axitinib as second line and beyond in metastatic clear cell renal cell carcinoma. Patients were treated with high doses of SLM, as high as 4 mg, twice daily for 2 weeks then once a day in combination with axitinib, without a dose limiting toxicity (DLT), and yet with promising efficacy signal [176]. A limitation of this study is that SLM can be incorporated into proteins as a replacement of methionine, especially in plasma [177]. This has the potential to limit the amount of Se that reach target organs or tumor tissues.

##### 4.1. Normal tissue protection by selenium

Selenium can also be used as an adjuvant for chemotherapy and radiation therapy [178,179]. Muecke et al., suggested that Se could restore selenoenzyme activity in Se-deficient patients that are under treatment. This would alleviate the side-effects of the treatment. In their phase 3 clinical trial, Se successfully reduced radiation therapy induced side effects while not impacting the efficacy of the antitumor effect of the therapy. In 2014, a follow-up research article was published where an increased 10-year survival rate was reported [179]. Because this study was conducted with selenium deficient subjects, it is likely that the se-dependent antioxidant enzymatic activities were restored, as is shown in cell culture systems [180]. Other mechanisms have not been identified, since these relatively low doses of sodium selenite do not

exhibit any toxicity in humans. While the biochemical of this incorporation is not well understood, there are suggestions that selenium supplementation is capable of protecting normal tissues against oxidative distress associated with therapy [181].

#### 5. Vitamin C, ascorbate

Since its discovery in 1933, vitamin C (ascorbic acid, AscH<sup>-</sup>) has been biologically implicated in a variety of applications [182,183]. Vitamin C is a ketolactone with two ionizable hydroxyl groups at the second and third carbons. The hydroxyl groups have pK<sub>a</sub>'s of 4.2 and 11.6, making the ascorbate monoanion (AscH<sup>-</sup>) the most prevalent species at pH of 7.4 [183]. AscH<sup>-</sup> acts as a reducing agent and donor antioxidant that can undergo two consecutive one-electron oxidations to produce the ascorbate radical (Asc<sup>•</sup>) and dehydroascorbic acid (DHA), respectively. The ability to undergo redox reactions makes vitamin C a potent antioxidant and pro-oxidant, depending on concentration and environment. Primates and domestic guinea pigs are the only species, including plants, that cannot synthesize vitamin C. This phenomenon is due to an inability to convert L-gluconolactone to L-ascorbic acid and contributes to humans' need to consume vitamin C through dietary supplementation [184].

##### 5.1. Cancer prevention

Because of vitamin C's ability to act as a potent antioxidant, vitamin C oral supplements were speculated to prevent cancer initiation. In 2004, researchers reported on the efficacy of 120 mg vitamin C supplementation for the prevention of cancer incidence, describing a randomized, double-blind, placebo-controlled primary prevention trial of 13,017 French adults. After a median time of 7.5 years, the subjects taking vitamin C supplements had lower total cancer incidence compared to controls [185]. Conversely, this same cohort had a higher incidence of skin cancer [186]. Another study, its results published in 2006, monitored 29,361 men for up to 8 years and found no evidence that vitamin C supplementation reduced the incidence of prostate cancer [187]. Additionally, a 2015 meta-analysis of seven randomized trials in which participants received either vitamin C or placebo did not show any evidence to support oral vitamin C supplementation prevents esophageal and gastric cancers [188]. However, these studies we not stratified according to circulating levels of ascorbate and therefore might not reflect what supplementation would do in a setting where the subjects were deficient.

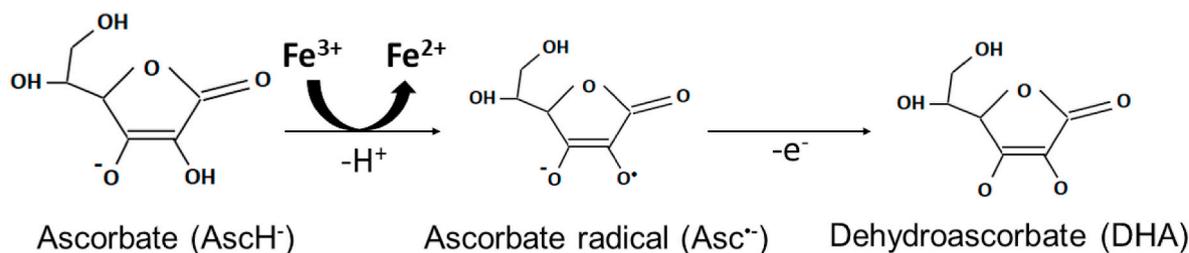
##### 5.2. Anticancer effects: ascorbate, a pro-oxidant

The anticancer mechanisms of ascorbate have been well elucidated in comparison to other redox active small molecules. Ascorbate oxidation produces hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and has long been proposed to enhance H<sub>2</sub>O<sub>2</sub>-mediated tumor cell killing [189–192]. Ascorbate oxidation occurs more readily in the presence of catalytic metals, including redox active iron, where ascorbate can act as a one-electron reducing agent and thereby reduce ferric (Fe<sup>3+</sup>) to ferrous (Fe<sup>2+</sup>) while producing an ascorbate radical (Equation (1), Fig. 4). The dysfunctional mitochondrial ETC of cancer cells are more prone to increased one electron reductions of O<sub>2</sub> to form superoxide (O<sub>2</sub><sup>•-</sup>) formation, which in turn increases catalytically active iron [193]. Thus, catalytically active iron is central to pharmacological ascorbate toxicity, as evidenced by the ability for desferrioxamine, an iron chelator, to mitigate cancer cell sensitivity to ascorbate [193,194].



In the presence of oxygen, the ferrous iron resulting from the reaction with ascorbate can be oxidized to generate superoxide (Equation (2)).





**Fig. 4.** Schematic oxidation of ascorbate. Ascorbate oxidation is enhanced by the presence of catalytically active ferric iron ( $\text{Fe}^{3+}$ ) that can readily accept an electron to be reduced to ferrous iron ( $\text{Fe}^{2+}$ ).

The superoxide radical can then be dismuted by superoxide dismutase (SOD) to produce  $\text{H}_2\text{O}_2$  and  $\text{O}_2$  (Equation (3)).



$\text{H}_2\text{O}_2$  is the central cytotoxic product of high-dose ascorbate. This large flux of  $\text{H}_2\text{O}_2$  generated after administration of pharmacological ascorbate (intravenous, typically tens of grams ascorbate) is then able to re-reduce ferric iron formed in reactions 2 and 4, setting up a detrimental redox cycle. The oxidants formed by the oxidation of pharmacological ascorbate can facilitate the release of even more iron into the labile iron from iron sulfur cluster-containing proteins (e.g. aconitase).

The  $\text{H}_2\text{O}_2$  produced is also able to rapidly interact with catalytically active ferrous iron in classical Fenton chemistry (Equation (4)) to produce the highly reactive hydroxyl radical ( $\text{HO}^\bullet$ ) and cause further cellular damage.



This mechanism of  $\text{H}_2\text{O}_2$ -mediated cytotoxicity is supported by the ability of catalase to decrease the amount of iron in catalytically active iron pools, and to mitigate the toxicity of pharmacological ascorbate by the removal of the  $\text{H}_2\text{O}_2$  formed [193,195]. Across multiple tumor cell lines, the  $\text{ED}_{50}$  of pharmacological ascorbate is directly correlated with intracellular catalase activity, whereas catalase inhibition with 3-amino-1,2,4-triazole enhanced ascorbate cancer cell killing [195]. These data all point to ascorbate being a pro-oxidant by the formation of high fluxes of  $\text{H}_2\text{O}_2$  upon its oxidation. The failure of high-dose ascorbate to show cytotoxic effects may be related to enhanced  $\text{H}_2\text{O}_2$  removal in untransformed cells *via* catalase and especially peroxiredoxins [195–201].

### 5.3. Pharmacological ascorbate therapy

In the 1970s, the potential utility of supraphysiological doses of ascorbate (pharmacological ascorbate; achieving mM plasma ascorbate concentrations) given intravenously was established in multiple trials that showed the safety and efficacy of the treatment with various terminal cancer patients [202–205]. In contrast, two randomized, double-blind clinical trials with high-dose oral ascorbate failed to show a clinical benefit relative to placebo [206,207]. These failures significantly reduced interest in using pharmacologic ascorbate as an anti-cancer agent.

Subsequent studies showed that oral ascorbate administration does not achieve the mM plasma ascorbate concentrations necessary to provide anticancer effects. Following an oral dose of 200 mg, the plasma ascorbate concentration reaches  $\approx 80 \mu\text{M}$  [208,209]. Following oral administration, ascorbate concentrations saturate at  $\approx 220 \mu\text{M}$  at doses  $\geq 1000 \text{ mg}$  [144,145]. Compare these to an intravenous ascorbate dose of 50 g, which can achieve plasma concentrations of 13.4 mM [210]. In a pilot clinical study, a 10 g intravenous dose of ascorbate reached an average plasma concentration of 1.1 mM [211]. Therefore, we currently think the most optimal way to effectively deliver pharmacological doses of ascorbate is intravenously.

Since determining efficacious delivery approaches, the field has seen

a resurgence in interest for evaluating the potential anticancer capabilities of pharmacological ascorbate (Table 4). In 2004, Riordan et al. performed a pilot study of pharmacological ascorbate in late-stage cancer patients diagnosed with renal cell carcinoma, colorectal cancer, pancreatic cancer, non-Hodgkin's lymphoma, and breast cancer [147, 148]. This study showed that gram dose intravenous ascorbate was safe and efficacious. The most common adverse events included nausea, edema, and dry mouth/skin. A 2008 phase I clinical trial using single-agent pharmacological ascorbate showed no significant toxicity but also failed to show any treatment responses [213]. The study concluded that although pharmacological ascorbate may be well tolerated, pharmacological ascorbate may be more beneficial as an adjuvant therapy rather than a single agent [213]. In 2014, a phase I clinical trial of 14 stage IV pancreatic cancer patients combined pharmacological ascorbate with gemcitabine to assess safety and efficacy [214]. The average overall survival was  $15 \pm 2$  months, and the average time to progression was  $26 \pm 7$  weeks as compared to 6 months and 9 weeks, historically [214]. Also in 2014, a phase I clinical trial in ovarian cancer showed that pharmacological ascorbate enhanced chemosensitivity in murine models and protected against carboplatin and paclitaxel chemotherapy-associated toxicity in human subjects [215]. A recent phase I clinical trial of newly diagnosed glioblastoma patients assessed the potential efficacy of pharmacological ascorbate with radiation and temozolomide [216]. This study reported a median overall survival of 18 months and a progression-free survival of 9.4 months, compared to the historical median overall survival of 14.6 months and progression-free survival of 6 months [217]. A phase I clinical trial in locally advanced pancreatic cancer that combined pharmacological ascorbate with therapeutic ionizing radiation and gemcitabine chemotherapy demonstrated a significant improvement in progression-free overall survival from 4.6 months to 13.7 months, relative to patients receiving radiation and gemcitabine alone [218].

Aside from the enhancement of cancer therapy, in a preclinical model pharmacological ascorbate ( $4 \text{ g kg}^{-1}$  delivered intraperitoneally) was shown to mitigate radiation- and chemotherapy-induced intestinal damage [221]. Similarly, pharmacological doses of ascorbate in C57Bl/6NHsd mice prevented radiation-induced (15 Gy/1 fx) alopecia and achromotrichia [221]. These exciting observations suggest that in addition to sensitizing cancer cells to radiation and chemotherapy, pharmacological ascorbate may also ameliorate radiation-induced side effects.

## 6. Conclusions

The promising, preliminary translational results summarized above have led to clinical studies of the aforementioned small redox active molecules based on fundamental differences in oxidative metabolism between cancer *versus* normal tissues. There are still many unknown mechanistic details needed to better understand the electron movement and relationships between anti-cancer and normal tissue effects of these molecules. However, these molecules are safe and well-tolerated in humans at supraphysiological levels and are therefore excellent candidates for interventional studies. Of particular interest is the ability of

**Table 4**

Recently completed clinical trials using pharmacological ascorbate as an adjuvant therapy.

Cancer Type	Dosage	Combination of Drugs	Results	References
Refractory Multiple Myeloma	1 g ascorbate on days 1, 4, 8, and 11 of a 21-day cycle for a maximum of 8 cycles IV	Ascorbate, Bortezomib and Arsenic Trioxide	Objective responses were observed in 27% of patients (2 partial and 4 minor).	[219]
Lymphoid Malignancies, Relapsed and Refractory	1000 mg ascorbate for 5 days during week 1 followed by twice weekly during weeks 2–6 IV	Ascorbate, Arsenic Trioxide	Overall median survival was 7.6 months	[220]
Advanced Stage Non-small Cell Lung Cancer	1 cycle is 21 days: IV pharmacological ascorbate (two 75 g infusions per week, up to 4 cycles)	Ascorbate, Carboplatin, paclitaxel, and ascorbate	6% complete and partial response	[193]
Glioblastoma	Ascorbate (from 15 to 125 g, 3 times per week for 7 weeks). Ascorbate (2 times per week, dose escalation until 20 mM plasma concentration, around ≈85 g infusion).	Ascorbate with radiation and temozolomide	Imaging confirmed partial responses to therapy (n = 4), stable disease (n = 9), and disease progression (n = 1). Progression-free survival 13.3 months; average overall survival 21.5 months	[193,216]
Late-stage Terminal Cancer Patients	150–710 mg/kg/day for up to 8 weeks	Ascorbate only	One patient had stable disease and continued treatment for 48 weeks. IV vitamin C was considered safe	[212]

redox active small molecules to function as antioxidants in normal untransformed cells while simultaneously functioning as cytotoxic pro-oxidants in neoplastic cells that can potentially both sensitize cancers to conventional therapies while protecting normal tissues opening new therapeutic windows of opportunity for improving clinical outcomes. A foundational example of this is pharmacological ascorbate, which takes advantage of dysfunctional mitochondria and increased labile iron pools in cancer cells, compared to normal cells, to generate cytotoxic fluxes of H<sub>2</sub>O<sub>2</sub>. This biphasic functionality may allow the field of cancer biology to further understand the underlying differences in electron movement in cancer cells relative to their normal cell counterparts. Due to their antioxidant/pro-oxidant potential, appropriate administration of melatonin, vitamin E, selenium, and vitamin C may offer novel, alternative strategies to enhance traditional cancer therapies, that take advantage of fundamental differences in oxidative metabolism between tumor and normal tissue.

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### Declaration of competing interest

The authors declare no conflict of interest.

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