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Hypothesis Papers

Potential use of n-3 PUFAs to prevent oxidative stress-derived ototoxicity caused by platinum-based chemotherapy

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ABSTRACT

Platinum-based compounds are widely used for the treatment of different malignancies due to their high effectiveness. Unfortunately, platinum-based treatment may lead to ototoxicity, an often-irreversible side effect without a known effective treatment and prevention plan. Platinum-based compound-related ototoxicity results mainly from the production of toxic levels of reactive oxygen species (ROS) rather than DNA-adduct formation, which has led to test strategies based on direct ROS scavengers to ameliorate hearing loss. However, favorable clinical results have been associated with several complications, including potential interactions with chemotherapy efficacy. To understand the contribution of the different cytotoxic mechanisms of platinum analogues on malignant cells and auditory cells, the particular susceptibility and response of both kinds of cells to molecules that potentially interfere with these mechanisms, is fundamental to develop innovative strategies to prevent ototoxicity without affecting antineoplastic effects. The n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs) have been tried in different clinical settings, including with cancer patients. Nevertheless, their use to decrease cisplatin-induced ototoxicity has not been explored to date. In this hypothesis paper, we address the mechanisms of platinum compounds-derived ototoxicity, focusing on the differences between the effects of these compounds in neoplastic versus auditory cells. We discuss the basis for a strategic use of n-3 PUFAs to potentially protect auditory cells from platinum-derived injury without affecting neoplastic cells and chemotherapy efficacy.

1. Introduction

Since the 1970s platinum compounds have been widely used to treat different types of cancer [1]. Despite their high effectiveness in controlling malignant tumors, their use is associated with several side effects including ototoxicity, which currently has no effective treatment or preventive strategy [1]. Among these drugs, cisplatin is the most representative compound, used to treat a variety of pediatric and adult malignancies [2].

Ototoxicity induced by platinum compounds commonly manifests as irreversible sensorineural hearing loss (HL). In the pediatric population, HL prevalence following platinum-based chemotherapy is not

well known, but has reported incidence as high as 90.1% [3]. Thus, it has been established that the pediatric population is at risk for the development of HL following cisplatin treatment, requiring long-term follow-up [3,4]. Moreover, HL in the pediatric population requires early intervention [4,5] because it may delay educational achievements and psychosocial development [4–6]. Among adults, up to 80% of patients may develop ototoxicity [1], decreasing quality of life in cancer survivors [7,8]. Thus, an otoprotective strategy against platinum compounds-related HL should be considered as an important outcome for improving patient prognosis [7], especially when there is limited access to hearing health and rehabilitation as it occurs in developing countries [9].

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In contrast to that occurring in tumor cells, where the platinum-based compounds exert their cytotoxic effects both by direct attack on DNA and massive ROS generation, on normal non-proliferative tissues such as auditory hair cells, the latter mechanism is the major cause of damage. Therefore, several otoprotective strategies have been attempted to reduce ototoxicity using antioxidant compounds. However, as increased ROS production also produces platinum-analogues anti-neoplastic effects, some of these strategies have interfered with anti-tumor efficacy.

In this hypothesis paper, we address the mechanisms of platinum compound-derived ototoxicity, the evidence accounting for the role of oxidative stress in this event and the differences between the effects of these antineoplastic substances in tumor and non-proliferative cells such as auditory hair cells. Thus, we discuss the basis to sustain the development of potential prophylactic strategies to ameliorate cisplatin-induced HL without interfering with antineoplastic activity, suggesting taking advantage of the indirect antioxidant properties of the n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs), whose safety has been proven in various clinical settings [10], including with cancer patients [11]. These compounds have been tested to reduce chemotherapy side effects [12–15], although their use to decrease cisplatin-induced ototoxicity has not been explored as yet.

2. Platinum-based chemotherapy agents

The different properties of platinum compounds are based on the distribution of their constituents. They have a central platinum atom, coordinated with two amino groups. Substituents of the active site are conformed by two covalent links to different electronegative motifs, including chlorine (cisplatin) and carboxylates (carboplatin and oxaliplatin) [2], thus providing them particular properties regarding their clinical effectiveness and side effects profile [2] (Fig. 1A).

Classically, the antineoplastic effect of platinum compounds is thought to be mediated through the production of nuclear DNA

adducts, leading to apoptosis [2] (Fig. 1B). Currently, there are different mechanisms reported to be behind cisplatin-induced cell death, including apoptosis, autophagy and others [16].

3. Platinum compounds-induced ototoxicity

Platinum compounds-induced ototoxicity clinically manifests as an irreversible, bilateral sensorineural HL, which could also be associated with tinnitus and vestibular disorders [1,4,17]. Symptoms onset may occur within hours to days following cisplatin administration [18], and even years after the completion of chemotherapy [19]. High-frequency HL typically occurs first [18] and it can progress to involve middle frequencies dose-dependently [18].

Platinum-based compounds have a limited entry to the inner ear [20], but these compounds damage some cochlear regions irreversibly [21]. Studies report auditory system cells tend to exhibit a special susceptibility to platinum compounds-induced apoptosis [22], including hair cells [23], stria vascularis, supporting cells and others [24], the first being one of the most affected [18] (Fig. 2). Marginal cell injury may also cause an impaired K^+ metabolism with subsequent dysfunction and loss of outer hair cells (OHC) [25], which is further aggravated by type I spiral ligament fibrocytes apoptosis triggered by cisplatin [26].

Platinum-based compounds have been observed to enter the cell through passive diffusion or by transporters [2]. Some transporters, such as copper transporters Ctr1 and Ctr2 and organic cation transporter OCT2, appear to play an important role in cisplatin ototoxicity and are exhibited in different auditory cells [27]. Precise details as to how these different transporters are involved in the ototoxicity remain unclear. However, it seems that different transporters may be responsible for varying degrees of cisplatin-related ototoxicity [27].

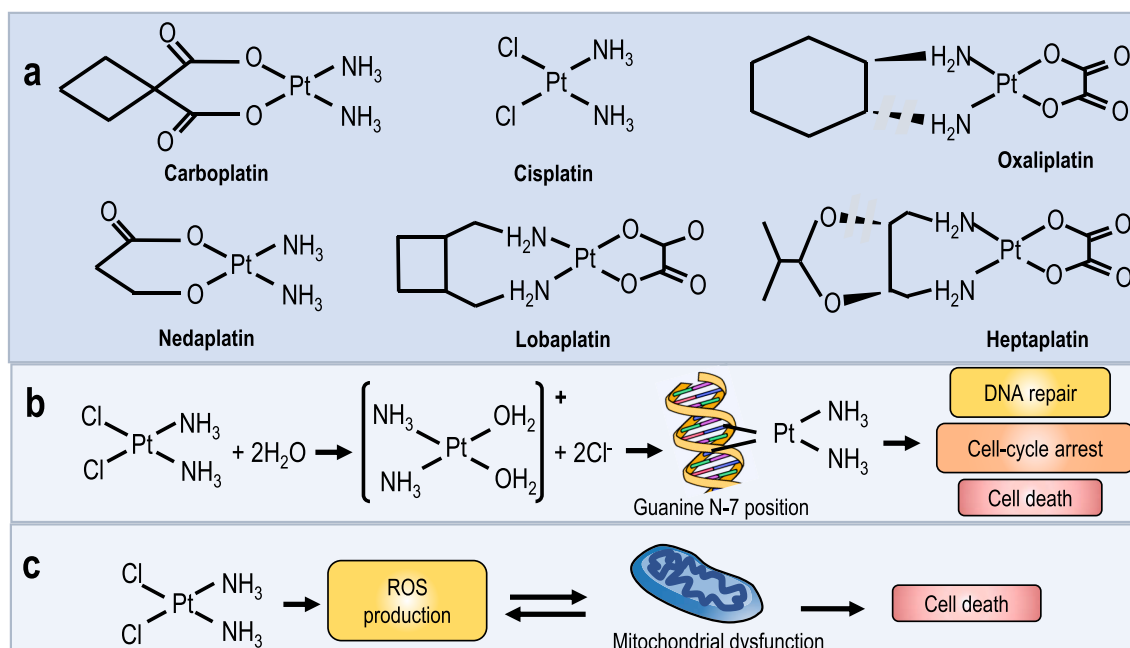


Fig. 1. Platinum analogues and their mechanisms of action.

(A) Platinum-based compounds are characterized by a central platinum atom coordinated with two amino-groups. Non-active substituents of this active site are conformed by two covalent links to symmetrical electronegative motifs, which confer different pharmacological profiles to these drugs. Platinum-based compounds have different ototoxic profiles, cisplatin being the most deleterious. (B) Non-active substituents of platinum analogues become displaced from platinum by water, forming highly reactive intermediates, which irreversibly binds to DNA at the N7 positions of purine bases, resulting in DNA-adduct formation. These adducts activate several signaling mechanisms such as DNA repair, cell cycle arrest, and apoptosis. (C) Platinum analogues lead to ROS production and also to mitochondrial dysfunction which enhances ROS generation, leading to cell death. Abbreviations: DNA = Deoxyribonucleic acid; ROS = Reactive Oxygen species.

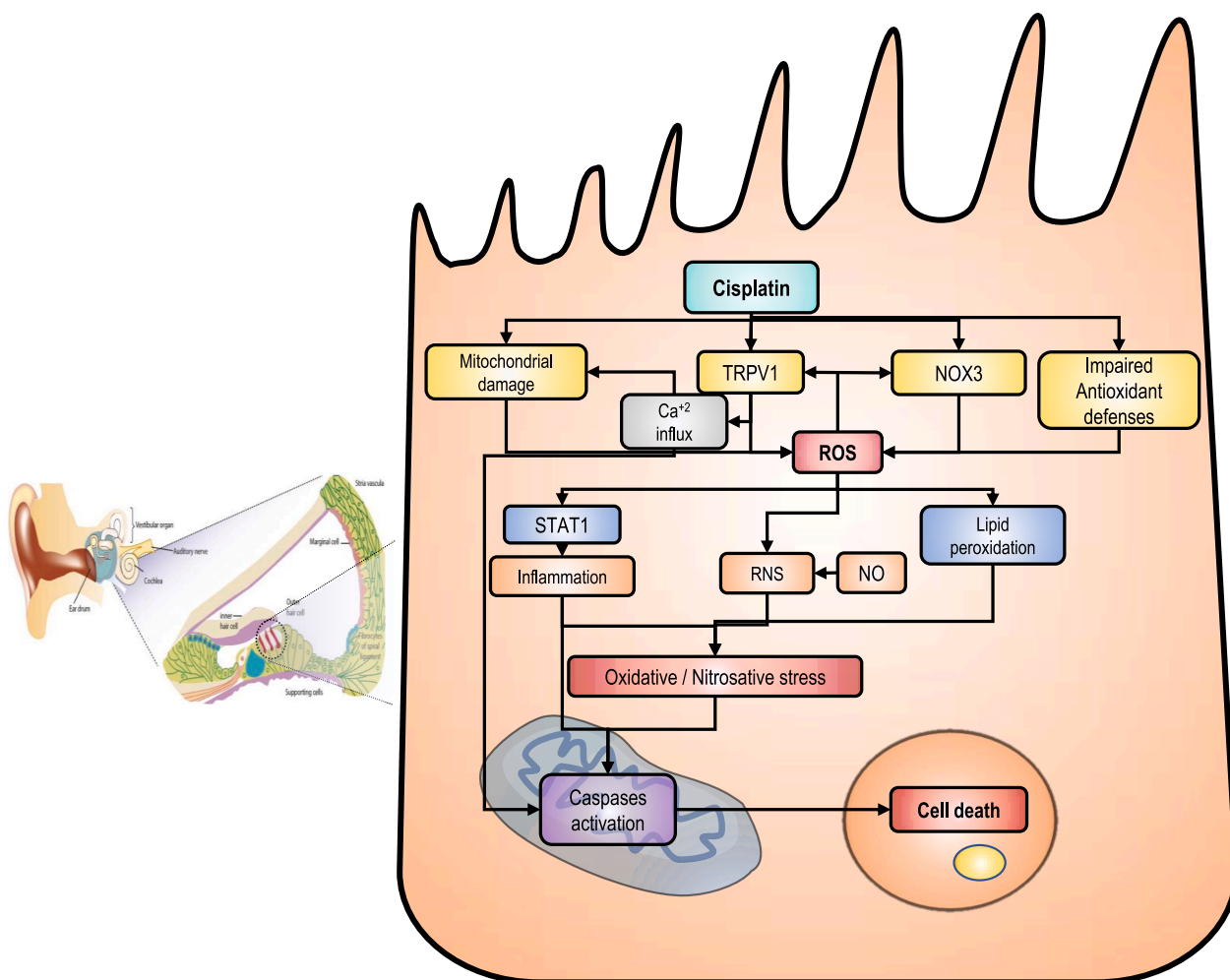


Fig. 2. Cisplatin and oxidative stress-mediated cell death in cochlear cells.

Different cochlear cells are affected by cisplatin treatment. We represented the major sources of ROS using an OHC. The ROS sources include cisplatin-induced mitochondrial damage that increases the production of mitochondrial ROS. Furthermore, the activation of TRPV1 and NOX3 would be a key event which trigger an increased ROS generation and an enhanced Ca^{+2} overload which is related to further TRPV1 and NOX3 activation, mitochondrial impairment and cell death. The ROS generation promotes lipid-peroxidation in cell membranes, decreases antioxidant defenses and leads to an activation of inflammatory pathways which can increase NO production, promote oxidative stress and trigger cell death. RNS could be formed by the reaction between ROS and NO leading to nitrosative stress, contributing to caspase activation and cell death of OHCs, stria vascularis, spiral ganglion and supporting cells. Abbreviations: ROS = Reactive Oxygen species; TRPV1 = transient receptor potential vanilloid 1 channel; NOX 3 = Nicotinamide adenine dinucleotide phosphate-oxidase 3; NO = Nitric oxide; STAT = Transcription factor signal transducer and activation of transcription 1; OHC= Outer hair cell.

3.1. Oxidative stress and direct mechanisms of ototoxicity

Reactive oxygen species (ROS) are normally present in low concentrations within cells; however, to prevent potentially detrimental oxidative action, cells have protective mechanisms such as antioxidant molecules and enzymes. Cochlear antioxidant systems include glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase [18]. When ROS production increase or the antioxidant defenses are impaired, oxidative stress increases, triggering biomolecule damage and cell death. Platinum-based chemotherapy has been shown to generate high amounts of ROS in cochlear cells, being a major mechanism of ototoxicity [18] (Fig. 2). These toxic amounts of ROS stem from different sources such as mitochondrial damage, increased activity of NOX3 (an isoform of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH)), lipid peroxidation, and antioxidant defense impairment. Moreover, ROS generation may lead to lipid peroxidation in cochlear tissues [28–30], triggering Ca^{+2} overload and activation of mitochondrial apoptosis signaling pathway with subsequent activation of caspases 3 and 9²². Interestingly, deletion of the p53-gene prevents cytochrome c translocation, caspase-3 activation,

and hair cell death, revealing a role of that mediator in mitochondrial ROS-derived apoptosis [31].

According to preclinical data, ROS production derived from NOX3 seems to be mediated by transient receptor potential vanilloid 1 channel (TRPV1) pathway [32], through a mechanism associated to cytosol Ca^{+2} overload [18,33], which triggers the activation of STAT1 (transcription factor signal transducer and activation of transcription 1) [34], leading to a caspase-induced OHC death [32]. STAT1 siRNA has been seen to abolish cisplatin-induced p53-activation, revealing that p53 also has a key role in this pathway [35].

Although direct nuclear DNA damage induced by platinum-based compounds has an important role in the cytotoxic effect, this mechanism cannot explain completely their high effectiveness as anticancer agents and their toxic effects exerted on normal non-proliferative tissues such as auditory hair cells. Thus, recent data based on cisplatin suggest that mitochondrial DNA damage plays a key role in the cytotoxic effects of platinum compounds [36,37]. Cisplatin may impair the electron transport chain function, increasing the production of ROS, which would be independent of the amount of nuclear DNA damage [37] (Fig. 1C).

Additionally, cumulative preclinical evidence indicates that NOX3, increases superoxide production following cisplatin-exposure [38–40]. This isoform is highly expressed in the inner ear [40] and its inhibition in an animal model by small interfering RNA confers protection to OHC, showing an important role in platinum compound-derived ototoxicity [41].

On the other hand, cisplatin may also enhance ROS production, impairing cochlear antioxidant defenses, probably due to reduced cochlear antioxidant enzyme activities or through antioxidant inactivation by increased ROS and organic peroxides production [18], for example by inhibition of the thioredoxin enzyme system [42]. Nitrosative stress also plays a significant role in cisplatin-induced ototoxicity. Increased ROS levels in the cochlear tissue can react with nitric oxide to generate peroxynitrite, an extremely reactive nitrogen species (RNS), which can modify proteins by S-nitrosylation [30].

The role of ROS and RNS in cisplatin-induced ototoxicity has drawn attention due to its relationship with protein S-nitrosylation and the subsequent effects on intracellular signaling pathways [43]. Studies have shown that cisplatin induces S-nitrosylation of cochlear LMO4, a transcriptional regulator controlling cell survival that is also a potential biomarker of cisplatin-induced oxidative inner ear damage [44]. Cisplatin-induced protein nitration generates downregulation of LMO4, enhancing caspases- and p53-mediated apoptosis in cochlear cells [44,45]. Rats treated with cisplatin had a significant shift in the amplitude of distortion product otoacoustic emissions (DPOAEs) of OHC [43]. This was associated with the S-nitrosylation of proteins located in the organ of Corti, stria vascularis, and spiral ganglions, known targets of cisplatin ototoxicity [43]. Thus, as is similar with ROS, RNS may react with various targets in the cell, causing cytotoxicity, although its mechanisms are less known than ROS and need to be more fully explored.

3.2. Indirect oxidative-mediated mechanisms of ototoxicity

Basic studies have reported that platinum-based compounds may also exert their cytotoxic effects by increasing the expression of pro-inflammatory mediators such as nuclear factor κ-B (NF-κB), tumor necrosis factor-α, interleukin-1β and -6, triggered by NF-κB activation, leading to apoptosis in the rat cochlea [1,46,47]. These effects are ameliorated in OHC following antioxidant treatment [48,49]. However, STAT1 activation also enhances inflammatory pathways, indirectly favoring cisplatin-induced irreversible hair cell damage by caspase-related mechanisms [32], revealing a cross-talk between the direct and

indirect oxidative stress-mediated cell death routes triggered by cisplatin [16]. Furthermore, it has also been reported that cisplatin can lead to cell death through autophagy in cultured auditory cells, a caspase-independent pathway related to increased mitochondrial-ROS production [50].

3.3. Risk factors for developing ototoxicity

Several factors may increase the risk of developing ototoxicity and HL following platinum-based chemotherapy. Cisplatin seems to be considerably more ototoxic than carboplatin and when patients receive both cisplatin and carboplatin, an increased rate of HL is reported [51]. The ototoxicity of other platinum compounds is less studied, but oxaliplatin seems to be less ototoxic than its predecessors [3]. Incidence of platinum-induced ototoxicity is dose-dependent [1,51] and elderly and pediatric populations are more susceptible to sensorineural HL following treatment [1,4,51]. Aging is one of the most important risk factors for developing malignancies but also for acquired HL [52]. Thus, it is important to consider and detect auditory deterioration triggered by platinum-based compounds in elderly patient follow-up as it can be difficult to differentiate from other causes of acquired HL such as age-related HL [53], or even contribute to other causes of auditory deterioration [17,52]. Radiation therapy (RT) is an important antitumor treatment and it is another significant ototoxicity risk factor when auditory structures are included in the radiation field [54]. RT triggers DNA damage of cochlear cells and direct ROS production leading to cell death [55]. It has been described that both high cumulative and fractionated radiation doses, RT technique, and concomitant or sequential platinum analogue use, increases ototoxicity risk [54,56]. Alternatively, Lim et al. [57], reported that radio-chemotherapy based on low-dose chemotherapy (IV cisplatin 40 mg/m²) did not increase levels of F₂-isoprostanes (as an oxidative stress index) in blood and urine samples in patients with nasopharyngeal cancer. Based on a study of seven patients, the authors concluded that radiotherapy did not increase isoprostane urine levels. Nevertheless, the study was not designed to conclude that 40 mg/m² weekly do not cause oxidative stress. Furthermore, they used intensity-modulated radiation therapy, so radiation doses might have been lower, though their paper does not make this clear.

Cumulative data indicate that genetic factors may be involved in HL susceptibility following cisplatin treatment [1,58,59]. These include gene variants of enzymes related to cisplatin pharmacokinetics, pharmacodynamics and polymorphisms in genes for DNA-adduct repair

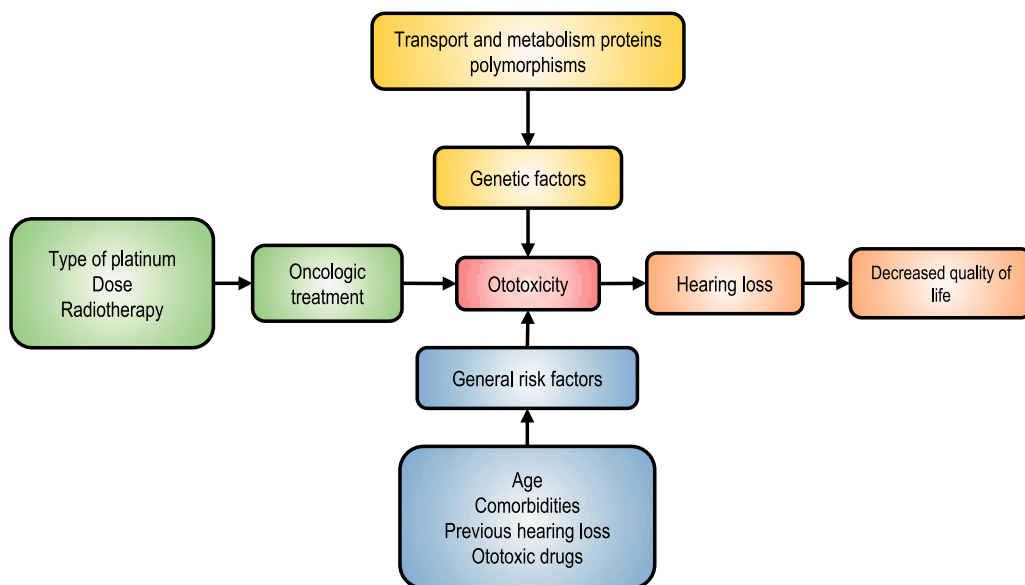


Fig. 3. A general view about platinum-analogues induced ototoxicity.

There are several factors which contribute to increase the risk of developing ototoxicity and subsequent hearing loss. Preserving life-quality of cancer survivors is an important outcome. Age, comorbidities and other general risk factors influence the possibility to develop hearing loss. Some gene polymorphisms of transport and metabolism protein are related to platinum-analogues uptake and detoxification. The oncologic treatment characteristics, for example, concomitant radiotherapy, remain as critical factors to develop ototoxicity.

Table 1
Clinical studies using antioxidants to ameliorate platinum analogue-induced ototoxicity.

Population	Study	Design	Neoplasm	Platinum drug	Additional treatment	Antioxidant	Groups	Results	
Pediatric	Brock et al. (2018) [65]	Multicenter, randomized, prospective trial	Standard-risk hepatoblastoma	Cisplatin 80 mg/m ²	-	STS 20 g/m ²² iv 6 h after Cisplatin	STS group n = 57 Cisplatin alone n = 52	Decreased risk of HL in STS group (RR 0.52; 95% CI 0.33–0.81; p = 0.002)	
	Freyer et al. (2017) [64]	Multicenter, randomized, open-label, phase 3 trial.	Different cancers treatable with cisplatin	Cisplatin ≥200 mg/m ² iv (according each patient disease)	-	STS 16 g/m ² iv 6 h after Cisplatin	STS group n = 49 -Observation group n = 55	Decreased likelihood of HL in STS group (OR 0.31 95% CI 0.13–0.73 p = 0.0036)	
	Gurney et al. (2014) [77]	Prospective	AR- or high-risk MB	Cisplatin 75 mg/m ² /cycle iv (≥ 4 cycles)	-RT -4 cycles of CTX, VCT, cisplatin and stem cell rescue	Amifostine 600 mg/m ² /dose iv. prior and 3 h into the cisplatin infusion	Amifostine-group n = 328 Control n = 51	Reduced risk of HL (OR:0.30 95% CI 0.14–0.64) in AR-patients (n = 263)	
	Katzenstein et al. (2009) [79]	Prospective randomized trial	Hepatoblastoma	-Cisplatin 100 mg/m ² iv (4–6 cycles) -Carboplatin 700 mg/m ² given iv (stage III-IV)	-5-fluorouracil 600 mg/m ² iv -VCT 1.5 mg/m ² iv	Amifostine 740 mg/m ² iv prior platinum agents	Cisplatin + 5-fluorouracil + VCT with (n = 30) and without (n = 34) Amifostine Cisplatin + Carboplatin with (n = 7) and without (n = 11) Amifostine	NS difference in ATs	
	Fouladi et al. (2008) [76]	Prospective	AR-MB	Cisplatin 75 mg/m ² /cycle iv (≥ 4 cycles)	-RT -CTX 4000 mg/m ² /cycle -VCT two 1.5 mg/m ² iv doses/cycle and stem cell rescue	Amifostine 600 mg/m ² /dose iv before and 3 h into the cisplatin infusion	-Amifostine-group, n = 52 -Control n = 35	Reduced need for hearing aids (> 25 dB loss at 2000 Hz) at one year (p = 0.005)	
	Marina et al. (2005) [80]	Prospective	Advanced PGCT	Cisplatin 40 mg/m ² /day	-Bleomycin 15 IU/m ² iv -Etoposide 100 mg/m ² /day iv	Amifostine 825 mg/m ² /day iv prior cisplatin	-Amifostine-group n = 25 -Historical comparison group n = 74	NS difference in ATs	
	Fisher et al. (2004) [78]	Prospective	MB or primitive neuroectodermal tumor	Cisplatin 70 mg/m ² iv (8 cycles)	-RT -Lomustin 75 mg/m ² (8 cycles) -VCT 1.5–2 mg/m ² iv	Amifostine 1,000 mg/m ² iv prior and 4 h after cisplatin	Amifostine-group n = 11	NS difference in ATs	
	Adults	Dias et al. (2015) [82]	Pilot, Randomized, prospective double blind	Different cancers treatable with cisplatin	Cisplatin Cumulative dose: 50–300 mg/m ²	-	Gyngko biloba 761 extract 240 mg/day	-Gyngko biloba extract 761 n = 8 -Placebo n = 7	Control Group showed lower DPOAE mean amplitudes than treated group (p < 0.05)
		Doolittle et al. (2001) [62]	Prospective Phase I clinical trial	Malignant brain tumors	Carboplatin 400 mg/m ² ia monthly for up to 1 year	-CTX 330 mg/m ² /day iv -Etoposide 200 mg/m ² /day ia/iv -25% Mannitol	STS 16–20 g/m ² per dose iv	STS 2 h after carboplatin n = 24 STS 4 h after carboplatin n = 17 -Control group: carboplatin n = 19 Untreated ears vs treated ears n = 11	-Decreased ATs at 8000 (p = 0.001) and 4000 Hz (p = 0.0075) -Delayed time to develop ototoxicity in the STS 4 h-group (p by log-rank test = 0.0018)
		Yoo et al. (2014) [83]	Pilot, Prospective randomized nonblinded open-label clinical trial	Advanced head and neck cancers	Cisplatin ≥100 mg/m ² iv (total cycles: 2–6)	RT	Transtympanic 2% NAC in 1 ear (other one was control)	NS difference in ATs	NS difference in ATs
Riga et al. (2013) [61]		Prospective randomized nonblinded open-label clinical trial	Different cancers treatable with cisplatin	Cisplatin Cumulative dose: 120–720 mg/m ²	-	Transtympanic 10% NAC in 1 ear (the other one was control)	Untreated ears vs treated ears n = 20	Decreased ATs in the treated ears at 8000 Hz (p = 0.005)	
Rolland et al. (2019) [84]		Prospective randomized controlled trial	Advanced head and neck cancers	Cisplatin cumulative dose: 233 ± 145 mg	RT	Transtympanic 25% STS + hyaluronate gel in 1 ear (the other one was control)	Untreated ears vs treated ears n = 13	NS difference in ATs	
<p>HL=Hearing loss. RT = Radiotherapy. STS = Sodium thiosulfate. NAC = N-Acetylcysteine. AR = Average risk. MB = Medulloblastoma. PGCT = Pediatric germ-cell tumors. CTX = Cyclophosphamide. VCT = Vincristine DPOAEs = Distortion product otoacoustic emissions. ATs = Auditory thresholds shift. IV = intravenous. IA = intraarterial. NS=No significant. OR = Odds ratio. RR = Relative Risk. CI: Confidence interval.</p>									

enzymes [58]. Other reported risk factors include [1]: noise exposure, kidney failure and some drugs such as aminoglycosides and furosemide [4,51] (Fig. 3).

4. Antioxidants and ototoxicity reduction

Numerous drugs and antioxidant strategies have been performed to ameliorate ROS-derived ototoxicity [60]. However, most of these studies have been performed in preclinical models, with clinical evidence remaining limited (Table 1).

As oxidative stress-associated injury may result from the imbalance between the production and the clearance of ROS/RNS by the antioxidant systems, major strategies avoiding oxidative stress in the inner ear include ROS detoxification by induction of antioxidant enzymes; ROS scavenging by nonenzymatic defenses; and inhibition of the downstream signaling pathways of a pathologic amount of ROS. Nonenzymatic antioxidants may be classified into directly acting antioxidants, which exert their antioxidant action via direct binding to ROS and RNS (e.g. scavengers and chain-breaking antioxidants); and indirectly acting antioxidants (e.g. chelating agents and n-3 PUFAs) that may develop antioxidant protection through binding to other molecules or inducing the expression of antioxidant systems (Fig. 4). However, it is important to consider that as platinum-based agents partially exert their antineoplastic effects by increased ROS production, antioxidant-based otoprotective strategies must be designed with extreme caution so as not to interfere with expected antitumor cytotoxicity. Furthermore, most preclinical interventions have been evaluated in non-cancer animal models. This fact limits the possibility of extrapolating these results to cancer patients, considering that cancer leads to multisystem alterations that may alter the effect of these interventions in the patient cochlea; and some interventions may potentially decrease chemotherapy effectiveness, critical to cancer treatment.

Clinical evidence of antioxidant strategies to prevent cisplatin-induced HL is still limited. In fact, several substances used as otoprotective agents, such as thiol compounds, have limited access to the cochlea because of the blood-inner ear barrier [61], requiring intratympanic administration. More importantly, several potential otoprotective agents are known to potentially interact with cisplatin, raising concerns about decreasing antitumor therapy efficacy [62,63].

The most encouraging results have been reported using thiol compounds. In a small pilot protocol in adults ($n = 36$) with malignant brain tumors treated on a monthly basis with intra-arterial carboplatin (total dose: 400 mg/m^2) and blood-brain barrier osmotic opening for up to 1 year, with ($n = 17$) and without ($n = 19$) systemic STS ($16\text{--}20 \text{ g/m}^2$), 4 h after each carboplatin cycle, noteworthy results were reported

[62]: 29% of patients exposed to STS developed ototoxicity, versus 84% in the control group ($p < 0.0018$). The same compound has also been evaluated in pediatric patients with different cancers in a multicenter, randomized, controlled, open-label, phase 3 trial [64]. Participants were randomly assigned to receive STS 16 g/m^2 intravenously 6 h after each cisplatin dose ($n = 49$) or not to receive STS following the chemotherapy ($n = 55$). After adjusting for stratification variables such as age (< 5 years or ≥ 5 years older), duration of cisplatin infusion (< 2 h or ≥ 2 h), and previous cranial irradiation, the risk of HL was lower in the STS group (OR 0.31; 95% CI 0.13–0.73; $p = 0.0036$). Unfortunately, participants with disseminated disease treated with STS had lower 3-year event-free survival (42%; 95% CI 0.21–0.61) versus control group (61%, 95% CI 0.39–0.77) (p value by log-rank test < 0.05) and a lower overall survival (45%, 95% CI 0.23–0.65) compared to control subjects (84%, 95% CI 0.62–0.94) (p value by log-rank test < 0.05). Therefore, despite the positive otoprotective results, the reported worsening of any oncologic primary endpoints makes complex recommending an STS-based strategy to reduce platinum analogue ototoxicity, even if only a small subgroup of patients had been affected.

Similarly, STS was tested in pediatric patients with standard-risk hepatoblastoma in a randomized multicenter clinical trial [65]. Patients were randomly allocated surgery and cisplatin (80 mg/m^2 over 6 h) ($n = 52$) or surgery, cisplatin and STS (20 g/m^2) over 15 min ($n = 57$), 6 h after the discontinuation of cisplatin for four preoperative and two postoperative courses. It was reported that patients who received STS had a lower incidence of HL of grade ≥ 1 (≥ 40 dB at frequencies ≥ 8000 Hz) (RR: 0.52; 95% CI 0.33–0.81; $p = 0.002$). After a median of 52 months of follow-up, no significant differences were reported in 3-year rates of event-free survival and overall survival. Although the two latter discussed studies have some similarities in methodology, their populations were different because the first study included children with different types of cancer and only patients with hepatoblastoma in the second study. Despite the positive otoprotective results, there is still no conclusive clinical evidence about STS interaction with platinum-based chemotherapy. Therefore, more clinical data is needed.

The decreased survival outcome reported in the study of Freyer et al. can be related to decreased chemotherapy efficacy, in turn resulting from a chemical interaction between STS and cisplatin [64]. This potential clinical interaction is consistent with several experimental studies, which reported significantly decreased cisplatin antitumor activity when thiols are used concomitantly [66–72]. Chemically, it has been described that thiol compounds bind to platinum molecules [73–75], decreasing their antitumor effect.

Amifostine is another thiol-based potential otoprotective molecule clinically tested with controversial results. Although several studies

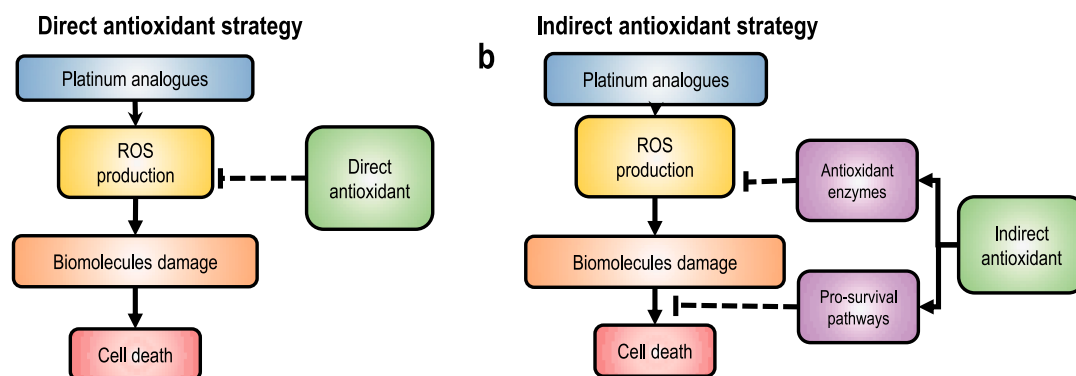


Fig. 4. Direct and indirect antioxidant strategies.

(A) Platinum analogues triggers ROS production which damages different molecules, leading to cell death. Direct antioxidant strategies aim to counteract this ROS production by direct binding to ROS and thus limiting their reaction with essential molecules, preventing cell death. As ROS production also mediates antitumor effects of platinum analogues, this strategy potentially may interfere with antitumor effects of cisplatin. Thus, a reduced antitumor efficacy may occur. (B) Indirect antioxidant strategies focus on the induction of pro-survival pathways and antioxidant enzymes which indirectly interfere on the deleterious effects triggered by the ROS production. Abbreviations: ROS = Reactive Oxygen species.

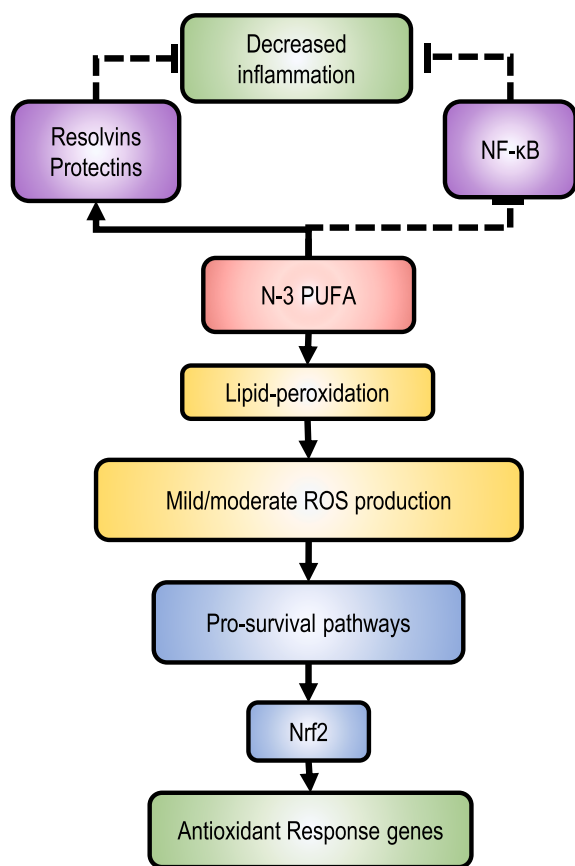


Fig. 5. Cytoprotective mechanisms of n-3 PUFAs.

The main proposed otoprotective mechanism of n-3 PUFAs would be related to a mild to moderate ROS production by lipid-peroxidation triggering pro-survival pathways activation such as Nrf2, which promotes an antioxidant response to oxidative insults. Furthermore, n-3 PUFAs incorporation to membrane triggers the formation of anti-inflammatory molecules such as resolvins and protectins as well as inhibiting the expression of NF- κ -B; a key pro-inflammatory molecule. Abbreviations: n-3 PUFAs = n-3 long-chain polyunsaturated fatty acids; Nrf2 = Nuclear factor erythroid 2-related factor 2; NF- κ -B = nuclear factor κ -B.

reported that amifostine have a protective effect against cisplatin-induced HL in pediatric patients, particularly with medulloblastoma [76,77], other studies failed to prove any otoprotective effect in the oncologic pediatric population [78–80] (Table 1). Thus, a meta-analysis of randomized controlled trials comparing platinum-based therapy with an otoprotective intervention or a placebo concludes that there is no evidence from individual studies in pediatric patients with osteosarcoma or hepatoblastoma that underscores the use of amifostine as an otoprotective intervention [8].

Another antioxidant tested is Ginkgo biloba extract, a polyphenolic mix with reported preclinical properties against cisplatin ototoxicity [81]. A double-blind pilot experience was performed in 15 cancer patients treated with cisplatin who were randomized to receive Ginkgo biloba extract-761 ($n = 8$) or a placebo ($n = 7$). After 3 months of follow-up, the control group showed smaller DPOAEs mean amplitudes and smaller signal/noise ratio than the supplemented group, which suggested that Ginkgo biloba extract-761 might have had an otoprotective effect against cisplatin ototoxicity [82].

As it has been suggested that systemic administration of otoprotective agents could affect platinum-based chemotherapy, local delivery to the ear through transtympanic administration of these otoprotective agents would circumvent the ototoxic effect, thereby maintaining otoprotective properties. Usage of transtympanic n-acetylcysteine has been reported in pilot trials at 2% ($n = 11$) [83] and 10% ($n = 20$) [61]

concentrations before systemic administration of cisplatin to have no significant otoprotection [83] and some benefit at 8000 Hz in treated ears compared to non-treated ones [61], respectively. Similarly, transtympanic use of STS gel has been reported in 13 patients prior to cisplatin delivery with no significant otoprotective effect; however, the trial was stopped due to poor accrual [84]. Local drug delivery still has several drawbacks, including: anatomic variations and other ear and drug factors which complicate sustaining a constant drug concentration in the middle ear and the passage to the inner ear [85]; repeated administrations and ear pain, limiting use in children; the need for an otorhinolaryngologist or a trained clinician to perform it, thus limiting its clinical use. However, intensive research is ongoing about local drug delivery to the ear and this will probably provide some benefit in certain cases [86].

5. Basis for using OMEGA-3

5.1. General properties of omega-3

PUFAs are a family of fatty acids with two or more unsaturation in their carboxylic chain. Among them, omega-3 fatty acids are characterized by having their first double bond in the third carbon. Mammals cannot produce n-3 PUFAs, which is why they obtain their precursors from their diet. Nutritionally the main essential n-3 fatty acid is the linolenic acid, which can be converted through enzymatic action in the cell. However, this conversion is inefficient and does not always meet body requirements, requiring supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through diet or capsules [87]. Both EPA and DHA are essential for proper human health [10] and can be converted from several biologically active molecules [88], through the action of enzymes or their direct oxidation. Some of these bioactive molecules are, resolvins, prostaglandins, neuroprostanes, protectins, maresins, among others [88]. Thus, the indirect antioxidant properties of n-3 PUFAs are related to the generation of mild oxidative stress by their lipid peroxidation, which leads to antioxidant response induction, protecting cells against a potential further oxidative insult. This response would be mainly mediated by the nuclear factor erythroid 2-related factor-2 (Nrf2) pathway activation [89,90], a key regulator of the antioxidant response. Under normal conditions, Nrf2 has a low constitutive expression [91] and localizes in the cytoplasm, where it interacts with the repressor protein Kelch-like ECH-associated protein 1 (Keap1) leading to Nrf2 degradation in proteasomes [91]. In response to enhanced ROS production, Keap1 is released from Nrf2, triggering the translocation of Nrf2 to the nuclei. Subsequently, this factor binds to the antioxidant response element, increasing the expression of proteins involved in cellular defense against oxidative stress [92]. Other cytoprotective effects mediated by n-3 PUFAs would be related to the inhibition of NF- κ B proinflammatory pathway [90] (Fig. 5).

In addition to their antioxidant properties, n-3 PUFAs exert anti-inflammatory functions [93,94]. While the molecular mechanisms that govern these effects are not completely understood, n-3 PUFAs are recognized to inhibit the arachidonic-dependent production of pro-inflammatory lipids [93,95], pro-inflammatory cytokines [94] and intracellular pathways such as EGFR, PKC, MAPK, NF- κ B and AP-1 [96]. Many of these effects can be explained by the inhibition of the NF- κ B pathway [97]. Thus, for example, n-3 PUFAs impede the TNF- α transcription induced by LPS through the inhibition of NF- κ B [98]. Additionally, it has been proposed that the anti-inflammatory effects could be mediated by disruption of lipid raft-dependent inflammatory signaling [94,99] and activation of the peroxisome proliferator-activated receptor (PPAR)- γ [100,101].

As stated, there are other molecules associated to n-3 PUFAs with anti-inflammatory capacities. These are the resolvins, produced from EPA and DHA, and protectins and maresins, produced from DHA [94,102]. The biosynthesis of resolvins involve transcellular

interactions and the LOX and COX-2 activities [102]. Interestingly, resolvins synthesis increased in humans with a diet rich in EPA and DHA [103,104]. These molecules help to finish an inflammatory state by activation of G-coupled receptors [105,106], which in turn can decrease migration of neutrophils, production of proinflammatory cytokines [102] and initiate tissue repair and healing mechanisms [107]. Due to their importance in controlling inflammatory conditions such as those found in cancer, several years ago a type of resolvins known as neuroprostane was encapsulated in a nanoparticle to test its effect on a breast cancer cell line [108]. It was found that encapsulated neuroprostane inhibited cell proliferation more efficiently than a non-encapsulated one, probably due to its highly lipophilic nature, which could be stabilized by the nanoparticles. It has been suggested that most of the effects of neuroprostanes and resolvins could be mediated by an increase of heme-oxygenase-1 and Nrf2 expression [109–111].

5.2. Omega-3 against oxidative injury

Several data suggest a potential role of n-3 PUFA administration as a protective strategy against oxidative-derived injury in different human conditions, such as acquired HL [112–114] and cardiac ischemia-reperfusion without harmful effects [115,116].

Epidemiological studies have reported that increased n-3 PUFA consumption is associated with reduced risk of age-related HL [112,113]. A recent prospective cohort study including 65,215 women evaluated the link between self-reported HL and consumption of fish and n-3 PUFAs. The multivariable-adjusted relative risk for HL among women in the highest quintile of n-3 PUFA intake was significantly lower than in women in the lowest quintile (0.85; 95% CI 0.80–0.91; $p < 0.001$)¹¹⁴. These results from n-3 PUFA supplementation could be explained on the basis of its indirect antioxidant effects, anti-inflammatory properties [112,117,118]. Previous data suggest that n-3 PUFA oral supplementation could be a feasible strategy to prevent oxidative damage and it also may influence cochlear function positively, improving hearing outcomes.

5.3. Omega-3 in cancer-related models

To our knowledge, there are no studies that have investigated the potential benefit of n-3 PUFA administration in this context. However, the use of these lipids to prevent cardiotoxicity derived from anthracyclines-mediated oxidative stress [119] is currently being tested by our group (registered trial N°ISRCTN69560410) [120]. It has been proposed that both DHA and EPA can be integrated into cardiomyocyte cell membranes, inducing Nrf2 leading to a myocardial tissue preconditioning against a further potential oxidative insult [89].

The use of n-3 PUFAs as preconditioning agents in preclinical models improved histopathological appearance of myocardial tissue, reduced oxidative stress biomarkers, preserved mitochondrial function, inhibited NF-κB and iNOS, and increased antioxidant enzymes [121–124]. One preclinical and one clinical study have used DHA and EPA to specifically protect normal tissue from toxic side effects of platinum-based chemotherapy. In the preclinical study rats treated with DHA for 10 days, starting 3 days prior to the cisplatin injection, survived and further showed a recovery of glomerular flow rate [12]. In the clinical study, a randomized trial designed to reduce oxaliplatin-induced peripheral neuropathy was conducted in 71 patients with colon cancer. Patients were allocated a placebo or n-3 PUFAs at a daily dose of 1,244 mg coinciding with the start of chemotherapy until one month after the end of treatment. The n-3 PUFA-supplemented group ($n = 36$) showed a decreased incidence of peripheral neuropathy compared with the placebo group ($n = 35$) (OR 0.14; 95% CI 0.04–0.49, $p = 0.002$) without reported adverse effects [14]. In a similar manner, the use of DHA and EPA would enhance antioxidant defenses in order to prepare the cochlear tissue for the oxidative challenge derived from the use of platinum-based chemotherapy.

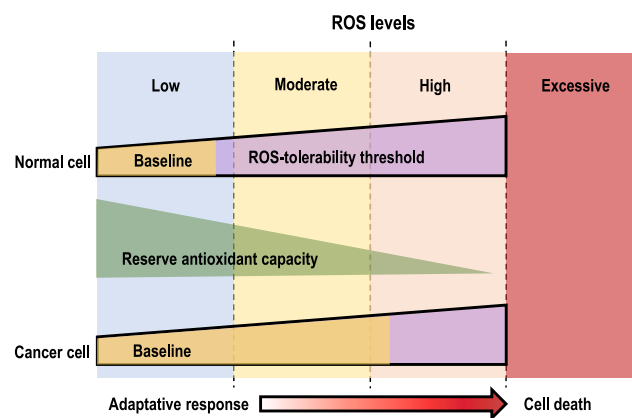


Fig. 6. A schematic representation of different ROS-threshold tolerability between cancer and normal cells

In this non-scaled representation, we showed that ROS production increases an adaptive response that it develops until ROS generation reaches a critical state, triggering cell death. Oncologic cells have higher basal ROS production by altered metabolism compared to normal cells which is accompanied by an increased antioxidant capacity in order to sustain their survival. Nonetheless, the reserve antioxidant capacity decreases as the ROS production increases which lead to normal cells have a higher antioxidant capacity to respond against an oxidative insult compared to cancer cell. Abbreviations: ROS = Reactive Oxygen species.

5.4. Omega-3 on normal and cancer cells

The use of n-3 PUFAs in cancer patients has drawn attention over time. Based on preclinical models, it has been suggested that EPA and DHA supplementation may reduce the tumor growth rate by sensitizing cancer cells to cellular death [125–127], increasing cancer cell susceptibility to conventional cytotoxic therapies with apparently no toxicity on normal cells [128–130]. However, not all omega-3 fatty acids have the same effects. High intake of the vegetal n-3 alpha-linolenic acid has been found to be associated with increased cancer risk development [131,132].

5.4.1. Omega-3 in cancer cells

The molecular pathways by which n-3 PUFAs would exert different effects in normal and some tumor cells are not fully understood. Nevertheless, there are several proposed mechanisms to explain this unequal response [133–135], including: differential n-3 PUFA incorporation rate into cell membranes, determining a different ROS production and tolerability to ROS levels (Fig. 6); modification of membrane lipid microdomains; modulation of eicosanoid metabolites; and altering gene expression by binding to nuclear receptors.

Compared to normal cells, tumor cells generate elevated basal levels of ROS [136] from different sources, including increased metabolism and pro-oxidant enzyme activity, relative hypoxia, oncogene activation, and endoplasmic reticulum stress [137–139]. These basal ROS levels are tightly balanced with a highly increased endogenous antioxidant capacity [140–142], in order to sustain its enhanced metabolic activity without altering its survival capability [134,140]. This enhanced antioxidant capacity could be explained by a highly increased constitutive expression of the Nrf2-pathway, an adaptive response to increase the basal tumor ROS-tolerability threshold and survival [143,144] (Fig. 6). The basally increased antioxidant capacity also needs an increased NADPH requirement [145,146], which in many cases is mediated by metabolic changes induced by KRAS oncogene [147]. Thus, as transformed cells have an increased Nrf2 activity and higher NADPH requirements compared with normal cells [145,146], neoplasms would be expected to have a lower margin to optimally handle additional oxidative insults [148]. An additional insult could be the pro-oxidant action of n-3 PUFA incorporation, which in preclinical models showed the

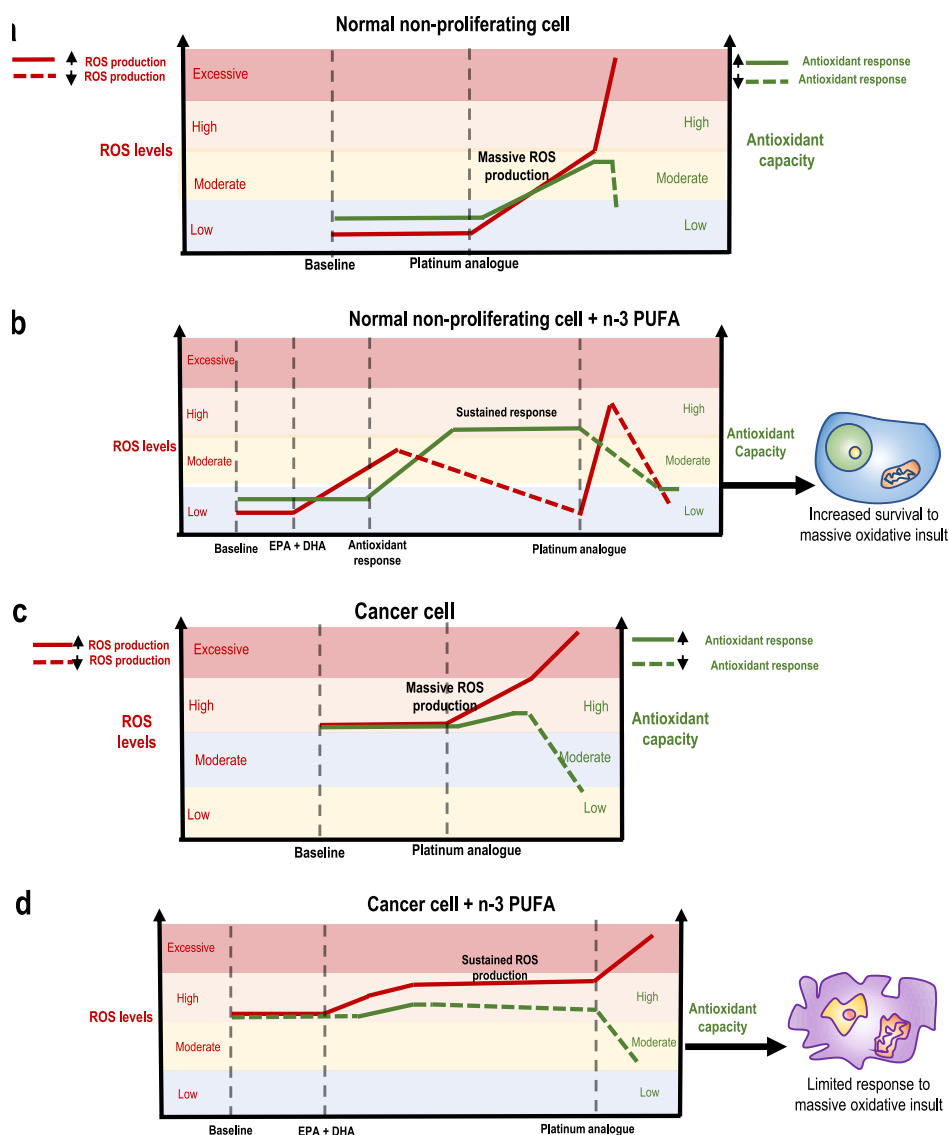


Fig. 7. Differential response of cancer and non-cancer cells to n-3 PUFAs supplementation following their exposure to platinum-based compounds

(A) How a normal non-proliferating cell would respond to platinum-analogue without n-3 PUFAs supplementation. At baseline, normal non-proliferating cells produce a low-level ROS which are controlled by antioxidant defenses. Platinum-analogue leads to a rapid increase in ROS production and a consumption of antioxidant defenses leading to an oxidative stress-mediated injury. (B) How a normal non-proliferating cell would respond to n-3 PUFAs supplementation. At baseline, normal cells produce a low-level ROS which are controlled by antioxidant defenses. The n-3 PUFAs incorporation develops an increase in ROS production by lipid-peroxidation which trigger an antioxidant response by pro-survival pathways activation. This nuclear-response is sustained until platinum analogue-derived oxidative challenge occurs. Platinum-analogue leads to a rapid increase in ROS production which is ameliorated by previously formed antioxidant response, limiting the harmful effects of ROS on biomolecules. (C) How a cancer cell would respond to platinum-analogue without n-3 PUFAs supplementation. At baseline, cancer cells produce high level of ROS which are controlled by antioxidant defenses due to an increased constitutive expression of pro-survival pathways such as Nrf2. Platinum-analogue leads to a rapid increase in ROS and a consumption of antioxidant defenses leading to uncontrolled oxidative injury. (D) How a cancer cell would respond to n-3 PUFAs supplementation. At baseline, cancer cells produce high level of ROS which are controlled by antioxidant defenses due to an increased constitutive expression of pro-survival pathways such as Nrf2. The n-3 PUFAs incorporation develops an increase in ROS production by lipid-peroxidation and a decrease in antioxidant potential. The oxidative insult triggered by n-3 PUFAs incorporation is sustained because basal production of ROS is augmented, the reserve antioxidant capacity is

diminished caused by constitutive antioxidant expression and high requirements of NADPH directed to sustain biomolecules synthesis during proliferation. Platinum-analogue leads to a rapid increase in ROS, however, cancer cell would be limited to respond against this oxidative challenge. Abbreviations: n-3 PUFAs = n-3 long-chain polyunsaturated fatty acids; ROS = Reactive oxygen species; Nrf2 = Nuclear factor erythroid 2-related factor 2; NADPH = nicotinamide adenine dinucleotide phosphate.

ability to act simultaneously by depleting tumor antioxidant defenses and inducing lipid peroxidation [149]. This pro-oxidative cascade would disrupt cancer cell redox homeostasis, leading to a nonlethal sustained pro-oxidative condition [134]. Thus, n-3 PUFA exposition might bring different cancer cell types near to their maximum oxidative stress tolerability, sensitizing them to a new massive ROS challenge, such as platinum-based chemotherapy [150–152] (Fig. 7). In a similar way, DHA can also be included rapidly into neoplastic mitochondrial membranes, increasing their susceptibility to oxidative stress and subsequent derived cell death [134].

Other proposed mechanisms include: the incorporation of n-3 PUFAs in cancer cell membranes would also alter the physical-chemical properties of bilipid layers as well as their lipid microdomain formation [153,154], favoring proapoptotic pathways in cancer cells rather than normal cells [133,155]; modification of eicosanoid metabolism of cancer cells, as a membrane enrichment with these lipids may reduce the activity of the cyclooxygenase (COX)-prostaglandin (PG)-E2 pathway, which is involved in tumor cell resistance to apoptosis [133,156,157]; binding to nuclear receptors such as peroxisome proliferator activating receptors in tumor cells, leading to up-regulation of

p53 protein, favoring caspase activation and apoptosis [126,158].

Therefore, neoplastic cells would be expected to have lower n-3 PUFA levels in their membranes compared to the non-transformed configuration [153,159]. This could be explained by a lower capability of transformed cells to synthesize EPA and DHA from their n-3 precursors and uptake them compared to normal cells, probably to protect themselves against n-3 PUFA-induced lipid peroxidation [160]. However, the lower PUFA uptake of cancer cells is still enough to generate an increased lipid-peroxidation rate when they are exposed to increased levels of EPA plus DHA [150,161]. Where common sense would indicate that the anti-inflammatory effect of PUFAs could enhance cancer cell resistance to chemo/radiotherapy, empirical evidence indicates that PUFAs can enhance cancer cell susceptibility to apoptosis, diminish angiogenesis and metastasis [162]. Alternatively, it has been reported that in human and animal models, omega-3 PUFAs can inhibit tumor infiltration of macrophages and T-suppressors while on the contrary they stimulate proliferation and activation of immune cells that decrease tumor growth [163–166]. Additionally, resolvins, which have been proposed to have an anti-proliferative effect on oral squamous cell carcinoma [167], interestingly, also inhibit cancer stem cell stemness

and epithelial-to-mesenchymal transition (EMT) in a hepatic carcinoma [168]. These anticancerogenic roles are probably mediated by their anti-inflammatory effects [167]. Thus, for example, resolving D1 inhibits TGF-1-induced EMT of a lung cancer cell line [169] and has been proposed as an immunotherapy enhancer [170]. Therefore, resolvins may also account for decreasing ototoxicity caused by platinum-based chemotherapy.

5.4.2. Omega-3 in normal cells

In contrast to most cancer cells, normal cells have the capacity to induce a higher margin of antioxidant activity to compensate oxidative insults, as their basal ROS production tend to be substantially less [171]. Despite this higher margin, these responses can also be overwhelmed by massive ROS production, so if the aggression is rapid, as occurs with platinum-based compounds where there is not enough time to generate responses that require gene expression [172]. In this case, there are no differences between the irreversible oxidative damage generated to normal and oncologic cells, stress that explains the observed cochlear cell death. However, in contrast to cancer cells, normal cells not previously exposed to n-3 PUFAs can be indirectly favored by the uptake of these fatty acids [160] due to their greater capacity to generate inducible antioxidant responses [137,171] (Fig. 7). This allows normal cells to adapt to the mild/moderate pro-oxidative effect of n-3 PUFA incorporation, which in turn grants resistance to further massive oxidative insults such as those caused by platinum-based compounds.

At the molecular level, when n-3 PUFAs are incorporated, a lipid-peroxidation cascade is triggered, leading to a mild-moderate intracellular oxidative stress leading to Nrf2 activation with the subsequent expression of antioxidant responses (Fig. 5). Because this gene expression is oversized and its effects are maintained over time, they provide protection to normal cells against an oxidative aggression generated by a future platinum compounds intervention. As most tumor cells have high constitutive expression of Nrf2 and elevated basal ROS production, the stimuli aimed to activate this transcription factor will tend to be ineffective. In addition, the higher incorporation rate of n-3 PUFAs in normal cells leads to increased production of cytoprotective molecules such as lipoxins, resolvins, and protectins, as well as decreased expression of NF- κ B [90,152,173,174], and may also defend normal cells against other anticancer treatments [175].

6. Otoprotective strategy based on OMEGA-3

Like other cells such as those of the retina and nervous system, the cells of the auditory system need an adequate supplementation of PUFAs for their proper functioning. Thus, for example, mice overexpressing an enzyme that convert n-6 to n-3 (Fat-1) are less affected by aging-induced loss of auditory function [176]. Similar results have been obtained in humans where a diet supplemented with omega-3 associated with lower risk of hearing loss in women [114] and auditory loss prevention in the elderly [112,113]. Moreover, use of omega-3 has been proposed to treat inner hearing disorders such as Meniere's disease [177]. One of the effects of omega-3 is likely through activation of prostanoid receptors which can regulate auditory blood flow, protection of sensory cells and immune responses in cochlea [178].

On the basis of the evidence discussed and results of studies using n-3 PUFA supplementation before the oxidative challenge [12,14,115], we hypothesize that it is possible to precondition patients' cochlear cells against platinum-derived oxidative damage through an indirect antioxidant intervention based on these lipids (Fig. 4). Theoretically, it would be possible to induce an upregulation of antioxidant enzymes and related pathways of cochlear cells, by a short-term administration of oral doses of n-3 PUFAs before platinum-based compound cycles, alone or combined with other treatments, in order to increase the capacity of these cells to resist the treatment-induced oxidative insult [89,133,175]. It is important to note that nerve cells are more prone to

uptake these lipids than other normal cells [179] and clinical trials that have used similar strategies to protect cardiomyocytes from ischemia-reperfusion injury [115], renal [12] and peripheral nerve tissue [14] from platinum-based compounds treatment, achieved successful prevention of oxidative-derived injury.

Additionally, proper use of n-3 PUFAs in cancer patients has proven to be safe, and no harmful effects have been reported during and after platinum- and non-platinum-based chemotherapies, while also not affecting oncologic treatment efficacy [14,119,127,133,134,180]. In this sense, n-3 PUFA supplementation in pediatric patients with lymphoblastic leukemia ($n = 32$) decreased methotrexate-induced hepatotoxicity compared to placebo ($n = 33$) ($p < 0.01$) without side effects [13]. Moreover, DHA can increase Nrf2 activity at least in breast cancer [181] and leukemia [182] cells mediating cancer cell apoptosis, suggesting that PUFAs can effectively protect normal cells and enhance cisplatin ROS-induced cell death. Therefore, n-3 PUFA supplementation would also be a feasible and safe strategy in pediatric patients.

7. Concluding remarks and perspectives

Platinum-based compounds, especially cisplatin, are widely used to treat different malignancies in adults and children. However, their use may be limited due to several major adverse reactions, including ototoxicity. Hearing loss is especially relevant because of its long-term high incidence and detrimental effect on survivor quality of life, especially in children.

Although ototoxicity is recognized as a significant side effect, the real impact of HL secondary to platinum-based chemotherapy exposure has not been well reported or measured. Irreversible HL is also frequently underrated during the oncologic follow-up as its detection can be delayed to post oncologic therapy completion or may be masked by the clinical deterioration of the patients. Furthermore, audition measurements are not commonly considered as an evaluation to perform on patients exposed to platinum-based compounds inside or outside clinical research. Therefore, most large clinical trials involving treatments that include platinum-based compounds have not reported HL as a usual adverse event. This situation enhances the chance to omit HL as a treatment-related adverse event and diminishes physician perception to educate or advise patients about it.

It is also crucial to consider that young patients treated with a curative aim, due to longer life exposition to platinum-based compounds, have a higher probability of developing audition deterioration and that risk must be explained to patients and parents carefully. Furthermore, limitations to hearing health access and auditory aids persist, especially in developing areas.

Therefore, preventing platinum compound-derived ototoxicity is a well-established goal. In recent decades it has driven research into molecular targets and compounds to ameliorate this injury. Increased production of ROS triggered by platinum compounds has been shown at different levels to account for their anticancer capacity, but it also plays a key role in the development and progression of ototoxicity. This has led to testing different strategies based mainly on direct antioxidants to ameliorate the harmful effects of ROS on auditory cells, showing favorable results in terms of otoprotection. However, they have also been associated with several complications, including a reduction of platinum-compound anticancer efficacy, rendering a potential clinical application of these strategies still controversial.

In the development of effective strategies to prevent platinum compound-related ototoxicity, it is important to consider that neoplastic proliferative cells are significantly different from non-proliferative auditory cells in terms of metabolism and response to several stimuli, including external molecules and oxidative stress response. Developing an intervention for reducing ROS-derived damage in non-proliferative auditory cells without impairing the oxidative injury that platinum-compounds trigger on cancer cells is a specific concern.

Based on current evidence, it is plausible to think that a strategy

designed to precondition auditory cells by indirectly increasing an endogenous antioxidant response before exposure to platinum-compounds could protect these cells from platinum-based chemotherapy oxidative injury. There is potential to propose that an indirect, well-designed strategy would not trigger the same antioxidant response in the cancer cell, thus avoiding decreasing the anticancer capacity of platinum-compounds reported in direct antioxidant-based interventions. In this regard, an n-3 PUFAs-based strategy would also have the advantage of having been tested on different oncologic settings without reported interference with chemotherapy effectiveness. Finally, to date there are no reports of clinical trials designed to explore the potential benefit of indirect antioxidant strategies based on n-3 PUFAs aimed to reduce ototoxicity incidence or severity derived from platinum-based chemotherapy.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

- [1] L.P. Rybak, D. Mukherjee, V. Ramkumar, Mechanisms of cisplatin-induced ototoxicity and prevention, *Semin. Hear.* 40 (2) (2019) 197–204, <https://doi.org/10.1055/s-0039-1684048>.
- [2] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, *Nat. Rev. Drug Discov.* 4 (4) (2005) 307–320, <https://doi.org/10.1038/nrd1691>.
- [3] J.W. van As, H. van den Berg, E.C. van Dalen, Platinum-induced hearing loss after treatment for childhood cancer, in: J.W. van As (Ed.), *Cochrane Database of Systematic Reviews*, John Wiley & Sons, Ltd, Chichester, UK, 2016, <https://doi.org/10.1002/14651858.CD010181.pub2>.
- [4] D.R. Freyer, P.R. Brock, K.W. Chang, et al., Prevention of cisplatin-induced ototoxicity in children and adolescents with cancer: a clinical practice guideline, *Lancet Child Adolesc Heal* 4 (2) (2020) 141–150, [https://doi.org/10.1016/S2352-4642\(19\)30336-0](https://doi.org/10.1016/S2352-4642(19)30336-0).
- [5] J.K. Bass, K.R. Knight, T.I. Yock, K.W. Chang, D. Cipkala, S.S. Grewal, Evaluation and management of hearing loss in survivors of childhood and adolescent cancers: a report from the children's oncology group, *Pediatr. Blood Canc.* 63 (7) (2016) 1152–1162, <https://doi.org/10.1002/pbc.25951>.
- [6] K.R.G. Knight, D.F. Kraemer, E.A. Neuwelt, Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development, *J. Clin. Oncol.* 23 (34) (2005) 8588–8596, <https://doi.org/10.1200/JCO.2004.00.5355>.
- [7] A. Khan, N. Mubdi, A. Budnick, et al., The experience of hearing loss in adult survivors of childhood and young adult cancer: a qualitative study, *Cancer* 126 (8) (2020) 1776–1783, <https://doi.org/10.1002/cncr.32698>.
- [8] S.E. Pearson, J. Taylor, P. Patel, D.M. Baguley, Cancer survivors treated with platinum-based chemotherapy affected by ototoxicity and the impact on quality of life: a narrative synthesis systematic review, *Int. J. Audiol.* 58 (11) (2019) 685–695, <https://doi.org/10.1080/14992027.2019.1660918>.
- [9] M.S. Harris, E.E. Dodson, Hearing health access in developing countries, *Curr. Opin. Otolaryngol. Head Neck Surg.* 25 (5) (2017) 353–358, <https://doi.org/10.1097/MOO.0000000000000392>.
- [10] P.C. Calder, Very long chain omega-3 (n-3) fatty acids and human health, *Eur. J. Lipid Sci. Technol.* 116 (10) (2014) 1280–1300, <https://doi.org/10.1002/ejlt.201400025>.
- [11] J. Yu, L. Liu, Y. Zhang, J. Wei, F. Yang, Effects of omega-3 fatty acids on patients undergoing surgery for gastrointestinal malignancy: a systematic review and meta-analysis, *BMC Canc.* 17 (1) (2017) 271, <https://doi.org/10.1186/s12885-017-3248-y>.
- [12] M.E. El-mesery, M.M. Al-gayyar, H.A. Salem, M.M. Darweish, A. El-Mowafy, A.M. El, Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides, *Cell Div.* 17 (2009) 1–17, <https://doi.org/10.1186/1747-1028-4-6>.
- [13] N.S. Elbarbary, E.A.R. Ismail, R.K. Farahat, M. El-Hamamsy, ω-3 fatty acids as an adjuvant therapy ameliorates methotrexate-induced hepatotoxicity in children and adolescents with acute lymphoblastic leukemia: a randomized placebo-controlled study, *Nutrition* 32 (1) (2016) 41–47, <https://doi.org/10.1016/j.nut.2015.06.010>.
- [14] A. Esfahani, Mohammad Hossein Somi, H. Ayromlou, et al., The effect of n-3 polyunsaturated fatty acids on incidence and severity of oxaliplatin induced peripheral neuropathy: a randomized controlled trial, *Biomark Res* 4 (1) (2016) 13, <https://doi.org/10.1186/s40364-016-0066-3>.
- [15] Z. Ghoreishi, A. Esfahani, A. Djazayeri, et al., Omega-3 fatty acids are protective against paclitaxel-induced peripheral neuropathy: a randomized double-blind placebo controlled trial, *BMC Canc.* 12 (1) (2012) 355, <https://doi.org/10.1186/1471-2407-12-355>.
- [16] E. Gentilin, E. Simoni, M. Candito, D. Cazzador, L. Astolfi, Cisplatin-induced ototoxicity: updates on molecular targets, *Trends Mol. Med.* 25 (12) (2019) 1123–1132, <https://doi.org/10.1016/j.molmed.2019.08.002>.
- [17] R.D. Frisina, H.E. Wheeler, S.D. Fossa, et al., Comprehensive audiometric analysis of hearing impairment and tinnitus after cisplatin-based chemotherapy in survivors of adult-onset cancer, *J. Clin. Oncol.* 34 (23) (2016) 2712–2720, <https://doi.org/10.1200/JCO.2016.66.8822>.
- [18] L.P. Rybak, D. Mukherjee, S. Jajoo, V. Ramkumar, Cisplatin ototoxicity and protection: clinical and experimental studies, *Tohoku J. Exp. Med.* 219 (3) (2009) 177–186, <https://doi.org/10.1620/tjem.219.177>.
- [19] A.M. Breglio, A.E. Rusheen, E.D. Shide, et al., Cisplatin is retained in the cochlea indefinitely following chemotherapy, *Nat. Commun.* 8 (1) (2017) 1654, <https://doi.org/10.1038/s41467-017-01837-1>.
- [20] G. Laurell, A. Andersson, B. Engström, H. Ehrsson, Distribution of cisplatin in perilymph and cerebrospinal fluid after intravenous administration in the Guinea pig, *Canc. Chemother. Pharmacol.* 36 (1) (1995) 83–86, <https://doi.org/10.1007/BF00685738>.
- [21] C. Schultz, P.H. Pecora Liberman, M.V. Schmidt Goffi-Gomez, Are there cochlear dead regions involved in hearing loss after cisplatin ototoxicity? *Audiol Neurotol* 24 (5) (2019) 253–257, <https://doi.org/10.1159/000502250>.
- [22] P. Devarajan, M. Savoca, M.P. Castaneda, et al., Cisplatin-induced apoptosis in auditory cells: role of death receptor and mitochondrial pathways, *Hear. Res.* 174 (1–2) (2002) 45–54 <http://www.ncbi.nlm.nih.gov/pubmed/12433395>.
- [23] V. Hellberg, I. Wallin, H. Ehrsson, G. Laurell, Cochlear pharmacokinetics of cisplatin: an in vivo study in the Guinea pig, *Laryngoscope* 123 (12) (2013) 3172–3177, <https://doi.org/10.1002/lary.24235>.
- [24] D. Ding, J. Zhang, H. Jiang, W. Xuan, W. Qi, R. Salvi, Some ototoxic drugs destroy cochlear support cells before damaging sensory hair cells, *Neurotox. Res.* (2020), <https://doi.org/10.1007/s12640-020-00170-8>.
- [25] G. Laurell, A. Ekborn, A. Viberg, B. Carlén, Effects of a single high dose of cisplatin on the melanocytes of the stria vascularis in the Guinea pig, *Audiol Neurotol* 12 (3) (2007) 170–178, <https://doi.org/10.1159/000099020>.
- [26] F. Liang, B.A. Schulte, C. Qu, W. Hu, Z. Shen, Inhibition of the calcium- and voltage-dependent big conductance potassium channel ameliorates cisplatin-induced apoptosis in spiral ligament fibrocytes of the cochlea, *Neuroscience* 135 (1) (2005) 263–271, <https://doi.org/10.1016/j.neuroscience.2005.05.055>.
- [27] S. Waissbluth, S.J. Daniel, Cisplatin-induced ototoxicity: transporters playing a role in cisplatin toxicity, *Hear. Res.* 299 (2013) 37–45, <https://doi.org/10.1016/j.heares.2013.02.002>.
- [28] L.P. Rybak, K. Husain, C. Morris, C. Whitworth, S. Somani, Effect of protective agents against cisplatin ototoxicity, *Am. J. Otol.* 21 (4) (2000) 513–520 <http://www.ncbi.nlm.nih.gov/pubmed/10912697>.
- [29] J.E. Lee, T. Nakagawa, T.S. Kim, et al., Role of reactive radicals in degeneration of the auditory system of mice following cisplatin treatment, *Acta Otolaryngol.* 124 (10) (2004) 1131–1135 <http://www.ncbi.nlm.nih.gov/pubmed/15768804>.
- [30] J.E. Lee, T. Nakagawa, T. Kita, et al., Mechanisms of apoptosis induced by cisplatin in marginal cells in mouse stria vascularis, *ORL J Otorhinolaryngol Relat Spec* 66 (3) (2004) 111–118, <https://doi.org/10.1159/000079329>.
- [31] A.G. Cheng, L.L. Cunningham, E.W. Rubel, Mechanisms of hair cell death and protection, *Curr. Opin. Otolaryngol. Head Neck Surg.* 13 (6) (2005) 343–348 <http://www.ncbi.nlm.nih.gov/pubmed/16282762>.
- [32] D. Mukherjee, S. Jajoo, K. Sheehan, et al., NOX3 NADPH oxidase couples transient receptor potential vanilloid 1 to signal transducer and activator of transcription 1-mediated inflammation and hearing loss, *Antioxidants Redox Signal.* 14 (6) (2011) 999–1010, <https://doi.org/10.1089/ars.2010.3497>.
- [33] J. Lu, W. Wang, H. Liu, H. Liu, H. Wu, Cisplatin induces calcium ion accumulation and hearing loss by causing functional alterations in calcium channels and exocytosis, *Am J Transl Res* 11 (11) (2019) 6877–6889. <http://www.ncbi.nlm.nih.gov/pubmed/31814894> Accessed March 1, 2020.
- [34] N.C. Schmitt, E.W. Rubel, N.M. Nathanson, Cisplatin-induced hair cell death requires STAT1 and is attenuated by epigallocatechin gallate, *J. Neurosci.* 29 (12) (2009) 3843–3851, <https://doi.org/10.1523/JNEUROSCI.5842-08.2009>.
- [35] T. Kaur, D. Mukherjee, K. Sheehan, S. Jajoo, L.P. Rybak, V. Ramkumar, Short interfering RNA against STAT1 attenuates cisplatin-induced ototoxicity in the rat by suppressing inflammation, *Cell Death Dis.* 2 (7) (2011) e180, <https://doi.org/10.1038/cddis.2011.63>.
- [36] F.M. Santandreu, P. Roca, J. Oliver, Uncoupling protein-2 knockdown mediates the cytotoxic effects of cisplatin, *Free Radic. Biol. Med.* 49 (4) (2010) 658–666, <https://doi.org/10.1016/j.freeradbiomed.2010.05.031>.
- [37] R. Marullo, E. Werner, N. Degtyareva, et al., Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions, *PLoS One* 8 (11) (2013) e81162, <https://doi.org/10.1371/journal.pone.0081162>.
- [38] D. Mukherjee, C.A. Whitworth, S. Nandish, G.A. Dunaway, L.P. Rybak,

- V. Ramkumar, Expression of the kidney injury molecule 1 in the rat cochlea and induction by cisplatin, *Neuroscience* 139 (2) (2006) 733–740, <https://doi.org/10.1016/j.neuroscience.2005.12.044>.
- [39] L.P. Rybak, D. Mukherjee, S. Jajoo, T. Kaur, V. Ramkumar, siRNA-mediated knock-down of NOX3: therapy for hearing loss? *Cell. Mol. Life Sci.* 69 (14) (2012) 2429–2434, <https://doi.org/10.1007/s00018-012-1016-3>.
- [40] B. Bánfi, B. Malgrange, J. Knisz, K. Steger, M. Dubois-Dauphin, K.-H. Krause, NOX3, a superoxide-generating NADPH oxidase of the inner ear, *J. Biol. Chem.* 279 (44) (2004) 46065–46072, <https://doi.org/10.1074/jbc.M403046200>.
- [41] D. Mukherjee, S. Jajoo, T. Kaur, K.E. Sheehan, V. Ramkumar, L.P. Rybak, Transtympanic Administration of Short Interfering (si)RNA for the NOX3 Isoform of NADPH Oxidase Protects Against Cisplatin-Induced Hearing Loss in the Rat, *Antioxidants Redox Signal.* 13 (5) (2010) 589–598, <https://doi.org/10.1089/ars.2010.3110>.
- [42] P. Dammeyer, V. Hellberg, I. Wallin, et al., Cisplatin and oxaliplatin are toxic to cochlear outer hair cells and both target thioredoxin reductase in organ of Corti cultures, *Acta Otolaryngol.* 134 (5) (2014) 448–454, <https://doi.org/10.3109/00016489.2013.879740>.
- [43] S. Jamesdaniel, S. Manohar, S. Hinduja, Is S-nitrosylation of cochlear proteins a critical factor in cisplatin-induced ototoxicity? *Antioxidants Redox Signal* 17 (7) (2012) 929–933, <https://doi.org/10.1089/ars.2012.4656>.
- [44] S. Jamesdaniel, R. Rathinam, W.L. Neumann, Targeting nitrative stress for attenuating cisplatin-induced downregulation of cochlear LIM domain only 4 and ototoxicity, *Redox Biol* 10 (2016) 257–265, <https://doi.org/10.1016/j.redox.2016.10.016>.
- [45] X. Zhou, M. Sang, W. Liu, et al., LMO4 inhibits p53-mediated proliferative inhibition of breast cancer cells through interacting p53, *Life Sci.* 91 (9–10) (2012) 358–363, <https://doi.org/10.1016/j.lfs.2012.08.005>.
- [46] H. So, H. Kim, J.-H. Lee, et al., Cisplatin cytotoxicity of auditory cells requires secretions of proinflammatory cytokines via activation of ERK and NF-kappaB, *J Assoc Res Otolaryngol* 8 (3) (2007) 338–355, <https://doi.org/10.1007/s10162-007-0084-9>.
- [47] D. Gao, H. Yu, B. Li, L. Chen, X. Li, W. Gu, Cisplatin toxicology: the role of pro-inflammatory cytokines and GABA transporters in cochlear spiral ganglion, *Curr. Pharmaceut. Des.* 25 (45) (2019) 4820–4826, <https://doi.org/10.2174/1381612825666191106143743>.
- [48] S.-J. Kim, C. Park, J.N. Lee, et al., Erdosteine protects HEI-OC1 auditory cells from cisplatin toxicity through suppression of inflammatory cytokines and induction of Nrf2 target proteins, *Toxicol. Appl. Pharmacol.* 288 (2) (2015) 192–202, <https://doi.org/10.1016/j.taap.2015.07.014>.
- [49] J. Kim, H.-J. Cho, B. Sagong, et al., Alpha-lipoic acid protects against cisplatin-induced ototoxicity via the regulation of MAPKs and proinflammatory cytokines, *Biochem. Biophys. Res. Commun.* 449 (2) (2014) 183–189, <https://doi.org/10.1016/j.bbrc.2014.04.118>.
- [50] H. Yin, Q. Yang, Z. Cao, et al., Activation of NLRX1-mediated autophagy accelerates the ototoxic potential of cisplatin in auditory cells, *Toxicol. Appl. Pharmacol.* 343 (2018) 16–28, <https://doi.org/10.1016/j.taap.2018.02.007>.
- [51] E. Clemens, A.C. de Vries, S.F. Pluijm, et al., Determinants of ototoxicity in 451 platinum-treated Dutch survivors of childhood cancer: a DCOG late-effects study, *Eur. J. Canc.* 69 (2016) 77–85, <https://doi.org/10.1016/j.ejca.2016.09.023>.
- [52] C.-H. Yang, T. Schrepfer, J. Schacht, Age-related hearing impairment and the triad of acquired hearing loss, *Front. Cell. Neurosci.* 9 (2015) 276, <https://doi.org/10.3389/fncel.2015.00276>.
- [53] J. Skalleberg, M.C. Småstuen, J. Oldenburg, T. Osnes, S.D. Fosså, M. Bunne, The relationship between cisplatin-related and age-related hearing loss during an extended follow-up, *Laryngoscope* (2020), <https://doi.org/10.1002/lary.28543>.
- [54] E.A.R. Theunissen, S.C.J. Bosma, C.L. Zuur, et al., D.W. Eisele (Ed.), *Sensorineural Hearing Loss in Patients with Head and Neck Cancer after Chemoradiotherapy and Radiotherapy: A Systematic Review of the Literature*, vol. 37, Head Neck, 2015, pp. 281–292, <https://doi.org/10.1002/hed.23551>.
- [55] M.A. Mujica-Mota, S. Lehnert, S. Devic, K. Gasbarrino, S.J. Daniel, Mechanisms of radiation-induced sensorineural hearing loss and radioprotection, *Hear. Res.* 312 (2014) 60–68, <https://doi.org/10.1016/j.heares.2014.03.003>.
- [56] A. Schuette, D.P. Lander, D. Kallogjeri, et al., Predicting hearing loss after radiotherapy and cisplatin chemotherapy in patients with head and neck cancer, *JAMA Otolaryngol - Head Neck Surg.* (2019), <https://doi.org/10.1001/jamaoto.2019.3550>.
- [57] K.H.C. Lim, C.Y.J. Lee, A. Earnest, R.C.S. Seet, B. Halliwell, Does radiotherapy increase oxidative stress? A study with nasopharyngeal cancer patients revealing anomalies in isoprostanes measurements, *Free Radic. Res.* 44 (9) (2010) 1064–1071, <https://doi.org/10.3109/10715762.2010.499906>.
- [58] D. Mukherjee, L.P. Rybak, Pharmacogenomics of cisplatin-induced ototoxicity, *Pharmacogenomics* 12 (7) (2011) 1039–1050, <https://doi.org/10.2217/pgs.11.48>.
- [59] E. Clemens, L. Broer, T. Langer, et al., Genetic variation of cisplatin-induced ototoxicity in non-cranial-irradiated pediatric patients using a candidate gene approach: the International PanCareLIFE Study, *Pharmacogenomics J.* (2019), <https://doi.org/10.1038/s41397-019-0113-1>.
- [60] S. Sheth, D. Mukherjee, L.P. Rybak, V. Ramkumar, Mechanisms of cisplatin-induced ototoxicity and otoprotection, *Front. Cell. Neurosci.* 11 (2017), <https://doi.org/10.3389/fncel.2017.00338>.
- [61] M.G. Riga, L. Chelis, S. Kakolyris, et al., Transtympanic injections of N-acetylcysteine for the prevention of cisplatin-induced ototoxicity, *Am. J. Clin. Oncol.* 36 (1) (2013) 1–6, <https://doi.org/10.1097/COC.0b013e31822e006d>.
- [62] N.D. Doolittle, L.L. Muldoon, R.E. Brummet, et al., Delayed sodium thiosulfate as an otoprotectant against carboplatin-induced hearing loss in patients with malignant brain tumors, *Clin. Canc. Res.* 7 (3) (2001) 493–500 <http://www.ncbi.nlm.nih.gov/pubmed/11297239>.
- [63] Y.J. Wu, L.L. Muldoon, E.A. Neuwelt, The chemoprotective agent N-acetylcysteine blocks cisplatin-induced apoptosis through caspase signaling pathway, *J. Pharmacol. Exp. Therapeut.* 312 (2) (2005) 424–431, <https://doi.org/10.1124/jpet.104.075119>.
- [64] D.R. Freyer, L. Chen, M.D. Krailo, et al., Effects of sodium thiosulfate versus observation on development of cisplatin-induced hearing loss in children with cancer (ACCL0431): a multicentre, randomised, controlled, open-label, phase 3 trial, *Lancet Oncol.* 18 (1) (2017) 63–74, [https://doi.org/10.1016/S1470-2045\(16\)30625-8](https://doi.org/10.1016/S1470-2045(16)30625-8).
- [65] P.R. Brock, R. Maibach, M. Childs, et al., Sodium thiosulfate for protection from cisplatin-induced hearing loss, *N. Engl. J. Med.* 378 (25) (2018) 2376–2385, <https://doi.org/10.1056/NEJMoa1801109>.
- [66] D.T. Dickey, Y.J. Wu, L.L. Muldoon, E.A. Neuwelt, Protection against cisplatin-induced toxicities by N-acetylcysteine and sodium thiosulfate as assessed at the molecular, cellular, and in vivo levels, *J. Pharmacol. Exp. Therapeut.* 314 (3) (2005) 1052–1058, <https://doi.org/10.1124/jpet.105.087601>.
- [67] D. Reser, M. Rho, D. Dewan, et al., L- and D- methionine provide equivalent long term protection against CDDP-induced ototoxicity in vivo, with partial in vitro and in vivo retention of antineoplastic activity, *Neurotoxicology* 20 (5) (1999) 731–748 <http://www.ncbi.nlm.nih.gov/pubmed/10591510>.
- [68] S. Aamdal, O. Fodstad, A. Pihl, Some procedures to reduce cis-platinum toxicity reduce antitumour activity, *Canc. Treat Rev.* 14 (3–4) (1987) 389–395 <http://www.ncbi.nlm.nih.gov/pubmed/3326670>.
- [69] S. Konstantinov, M. Topashka-Ancheva, M. Karaivanova, G. Zoneva, I. Galova, Antitumor, nephrotoxic and clastogenic effect of cis-DDP with DDTC or NAC, *Neoplasma* 41 (5) (1994) 253–258 <http://www.ncbi.nlm.nih.gov/pubmed/7854494>.
- [70] M. Inoue, C. Shimizu, H. Shimizu, O. Tanizawa, Neutralizing effect of sodium thiosulfate on antitumor efficacy of cisplatin for human carcinoma xenografts in nude mice, *Gynecol. Oncol.* 40 (1) (1991) 34–37 <http://www.ncbi.nlm.nih.gov/pubmed/1989912>.
- [71] Y. Iwamoto, T. Kawano, M. Ishizawa, K. Aoki, T. Kuroiwa, T. Baba, Inactivation of cis-diamminedichloroplatinum (II) in blood and protection of its toxicity by sodium thiosulfate in rabbits, *Canc. Chemother. Pharmacol.* 15 (3) (1985), <https://doi.org/10.1007/BF00263891>.
- [72] M. Viale, J.G. Zhang, I. Pastrone, M.A. Marigò, M. Esposito, W.E. Lindup, Cisplatin combined with tiopronin or sodium thiosulfate: cytotoxicity in vitro and antitumor activity in vivo, *Anti Canc. Drugs* 10 (4) (1999) 419–428 <http://www.ncbi.nlm.nih.gov/pubmed/10378678>.
- [73] J. Wang, R.V. Lloyd Faulconbridge, A. Fetoni, M.J. Guitton, R. Pujol, J.L. Puel, Local application of sodium thiosulfate prevents cisplatin-induced hearing loss in the Guinea pig, *Neuropharmacology* 45 (3) (2003) 380–393 <http://www.ncbi.nlm.nih.gov/pubmed/12871655>.
- [74] A. Ekbom, G. Laurell, P. Johnström, I. Wallin, S. Eksborg, H. Ehrsson, D-Methionine and cisplatin ototoxicity in the Guinea pig: D-methionine influences cisplatin pharmacokinetics, *Hear. Res.* 165 (1–2) (2002) 53–61 <http://www.ncbi.nlm.nih.gov/pubmed/12031515>.
- [75] B.W. Blakley, J.I. Cohen, N.D. Doolittle, et al., Strategies for prevention of toxicity caused by platinum-based chemotherapy: review and summary of the annual meeting of the Blood-Brain Barrier Disruption Program, Glendon Beach, Oregon, March 10, 2001, *Laryngoscope* 112 (11) (2002) 1997–2001, <https://doi.org/10.1097/00005537-200211000-00016>.
- [76] M. Fouladi, M. Chintagumpala, D. Ashley, et al., Amifostine protects against cisplatin-induced ototoxicity in children with average-risk medulloblastoma, *J. Clin. Oncol.* 26 (22) (2008) 3749–3755, <https://doi.org/10.1200/JCO.2007.14.3974>.
- [77] J.G. Gurney, J.K. Bass, A. Onar-Thomas, et al., Evaluation of amifostine for protection against cisplatin-induced serious hearing loss in children treated for average-risk or high-risk medulloblastoma, *Neuro Oncol.* 16 (6) (2014) 848–855, <https://doi.org/10.1093/neuonc/nbt241>.
- [78] M.J. Fisher, B.J. Lange, M.N. Needle, et al., Amifostine for children with medulloblastoma treated with cisplatin-based chemotherapy, *Pediatr. Blood Canc.* 43 (7) (2004) 780–784, <https://doi.org/10.1002/pbc.20132>.
- [79] H.M. Katzenstein, K.W. Chang, M. Krailo, et al., Amifostine does not prevent platinum-induced hearing loss associated with the treatment of children with hepatoblastoma: a report of the Intergroup Hepatoblastoma Study P9645 as a part of the Children's Oncology Group, *Cancer* 115 (24) (2009) 5828–5835, <https://doi.org/10.1002/cncr.24667>.
- [80] N. Marina, K.W. Chang, M. Malogolowkin, et al., Amifostine does not protect against the ototoxicity of high-dose cisplatin combined with etoposide and bleomycin in pediatric germ-cell tumors: a Children's Oncology Group study, *Cancer* 104 (4) (2005) 841–847, <https://doi.org/10.1002/cncr.21218>.
- [81] S.J. Choi, S.W. Kim, J.B. Lee, et al., Ginkgo biloba extracts protect auditory hair cells from cisplatin-induced ototoxicity by inhibiting perturbation of gap junctional intercellular communication, *Neuroscience* 244 (2013) 49–61, <https://doi.org/10.1016/j.neuroscience.2013.04.001>.
- [82] M.A. Dias, A.L.L. Sampaio, A.R. Venosa, E. de A. Meneses, C.A.C.P. Oliveira, The chemopreventive effect of Ginkgo biloba extract 761 against cisplatin ototoxicity: a pilot study, *Int. Tinnitus J.* 19 (2) (2015) 12–19, <https://doi.org/10.5935/0946-5448.20150003>.
- [83] J. Yoo, S.J. Hamilton, D. Angel, et al., Cisplatin otoprotection using transtympanic L-N-acetylcysteine: a pilot randomized study in head and neck cancer patients, *Laryngoscope* 124 (3) (2014) 1–8, <https://doi.org/10.1002/lary.24360>.
- [84] V. Rolland, F. Meyer, M.J. Guitton, et al., A randomized controlled trial to test the efficacy of trans-tympanic injections of a sodium thiosulfate gel to prevent

- cisplatin-induced ototoxicity in patients with head and neck cancer, *J Otolaryngol - Head Neck Surg.* 48 (1) (2019), <https://doi.org/10.1186/s40463-019-0327-x>.
- [85] A.N. Salt, S.K. Plontke, Principles of local drug delivery to the inner ear, *Audiol Neurotol* 14 (6) (2009) 350–360, <https://doi.org/10.1159/000241892>.
- [86] L.P. Rybak, A. Dhukhwa, D. Mukherjee, V. Ramkumar, Local drug delivery for prevention of hearing loss, *Front. Cell. Neurosci.* 13 (2019), <https://doi.org/10.3389/fncel.2019.00300>.
- [87] S.C. Cunnane, Problems with essential fatty acids: time for a new paradigm? *Prog. Lipid Res.* 42 (6) (2003) 544–568 <http://www.ncbi.nlm.nih.gov/pubmed/14559071>.
- [88] S.C. Dyall, Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA, *Front. Aging Neurosci.* 7 (APR) (2015), <https://doi.org/10.3389/fnagi.2015.00052>.
- [89] R. Rodrigo, M. Cereceda, R. Castillo, et al., Prevention of atrial fibrillation following cardiac surgery: basis for a novel therapeutic strategy based on non-hypoxic myocardial preconditioning, *Pharmacol. Ther.* 118 (1) (2008) 104–127, <https://doi.org/10.1016/j.pharmthera.2008.01.005>.
- [90] C. Cipollina, Endogenous generation and signaling actions of omega-3 fatty acid electrophilic derivatives, *BioMed Res. Int.* 2015 (2015), <https://doi.org/10.1155/2015/501792>.
- [91] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, *J. Biol. Chem.* 284 (20) (2009) 13291–13295, <https://doi.org/10.1074/jbc.R900010200>.
- [92] K. Taguchi, H. Motohashi, M. Yamamoto, Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution, *Gene Cell.* 16 (2) (2011) 123–140, <https://doi.org/10.1111/j.1365-2443.2010.01473.x>.
- [93] P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851 (4) (2015) 469–484, <https://doi.org/10.1016/j.bbalip.2014.08.010>.
- [94] P.C. Calder, Omega-3 fatty acids and inflammatory processes: from molecules to man, *Biochem. Soc. Trans.* 45 (5) (2017) 1105–1115, <https://doi.org/10.1042/BST20160474>.
- [95] L.D. Peterson, N.M. Jeffery, F. Thies, P. Sanderson, E.A. Newsholme, P.C. Calder, Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E2 production but have different effects on lymphocyte functions and cell-mediated immunity, *Lipids* 33 (2) (1998) 171–180, <https://doi.org/10.1007/s11745-998-0193-y>.
- [96] S.E. Lee, J.W. Lim, J.M. Kim, H. Kim, Anti-inflammatory mechanism of polyunsaturated fatty acids in *Helicobacter pylori*-infected gastric epithelial cells, *Mediat. Inflamm.* 2014 (2014), <https://doi.org/10.1155/2014/128919>.
- [97] C.J. Lo, K.C. Chiu, M. Fu, R. Lo, S. Helton, Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF- κ B activity, *J. Surg. Res.* 82 (2) (1999) 216–221, <https://doi.org/10.1006/jsre.1998.5524>.
- [98] T.E. Novak, T.A. Babcock, D.H. Jho, W.S. Helton, N.J. Espat, NF- κ B inhibition by ω -3 fatty acids modulates LPS-stimulated macrophage TNF- α transcription, *Am. J. Physiol. Lung Cell Mol. Physiol.* 284 (1 28–1) (2003), <https://doi.org/10.1152/ajplung.00077.2002>.
- [99] S.W. Wong, M.J. Kwon, A.M.K. Choi, H.P. Kim, K. Nakahira, D.H. Hwang, Fatty acids modulate toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner, *J. Biol. Chem.* 284 (40) (2009) 27384–27392, <https://doi.org/10.1074/jbc.M109.044065>.
- [100] B.M. Forman, J. Chen, R.M. Evans, Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ , *Proc. Natl. Acad. Sci. U. S. A.* 94 (9) (1997) 4312–4317, <https://doi.org/10.1073/pnas.94.9.4312>.
- [101] G. Krey, O. Braissant, F. L'Horsset, et al., Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay, *Mol. Endocrinol.* 11 (6) (1997) 779–791, <https://doi.org/10.1210/mend.11.6.0007>.
- [102] G. Bannenberg, C.N. Serhan, Specialized pro-resolving lipid mediators in the inflammatory response: an update, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1801 (12) (2010) 1260–1273, <https://doi.org/10.1016/j.bbalip.2010.08.002>.
- [103] E. Mas, K.D. Croft, P. Zahra, A. Barden, T.A. Mori, Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation, *Clin. Chem.* 58 (10) (2012) 1476–1484, <https://doi.org/10.1373/clinchem.2012.190199>.
- [104] A. Polus, B. Zapala, U. Razny, et al., Omega-3 fatty acid supplementation influences the whole blood transcriptome in women with obesity, associated with pro-resolving lipid mediator production, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861 (11) (2016) 1746–1755, <https://doi.org/10.1016/j.bbalip.2016.08.005>.
- [105] N. Chiang, J. Dallij, R.A. Colas, C.N. Serhan, Identification of resolvin D2 receptor mediating resolution of infections and organ protection, *J. Exp. Med.* 212 (8) (2015) 1203–1217, <https://doi.org/10.1084/jem.20150225>.
- [106] N. Chiang, X. de la Rosa, S. Libreros, C.N. Serhan, Novel resolvin D2 receptor Axis in infectious inflammation, *J. Immunol.* 198 (2) (2017) 842–851, <https://doi.org/10.4049/jimmunol.1601650>.
- [107] S.E. Headland, L.V. Norling, The resolution of inflammation: principles and challenges, *Semin. Immunol.* 27 (3) (2015) 149–160, <https://doi.org/10.1016/j.smim.2015.03.014>.
- [108] J. Roy, L.T. Oliveira, C. Oger, et al., Polymeric nanocapsules prevent oxidation of core-loaded molecules: evidence based on the effects of docosahexaenoic acid and neuroprostane on breast cancer cells proliferation, *J. Exp. Clin. Oncol. Res.* 34 (1) (2015), <https://doi.org/10.1186/s13046-015-0273-z>.
- [109] Y.Y. Lee, J.M. Galano, H.H. Leung, et al., Nonenzymatic oxygenated metabolite of docosahexaenoic acid, 4(RS)-4-F4t-neuroprostane, acts as a bioactive lipid molecule in neuronal cells, *FEBS Lett.* 594 (11) (2020) 1797–1808, <https://doi.org/10.1002/1873-3468.13774>.
- [110] D.G. Mostafa, H.H. Satti, Resolvin D1 prevents the impairment in the retention memory and hippocampal damage in rats fed a corn oil-based high fat diet by upregulation of Nrf2 and downregulation and inactivation of p66Shc, *Neurochem. Res.* 45 (7) (2020) 1576–1591, <https://doi.org/10.1007/s11064-020-03022-1>.
- [111] Z. Zhang, X. Hu, X. Qi, et al., Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice, *Mol. Vis.* 24 (2018) 274–285.
- [112] B. Gopinath, V.M. Flood, E. Rochtchina, C.M. McMahon, P. Mitchell, Consumption of omega-3 fatty acids and fish and risk of age-related hearing loss, *Am. J. Clin. Nutr.* 92 (2) (2010) 416–421, <https://doi.org/10.3945/ajcn.2010.29370.416>.
- [113] C. Dullemeijer, P. Verhoef, I.A. Brouwer, F.J. Kok, R.J.M. Brummer, J. Durga, Plasma very long-chain n-3 polyunsaturated fatty acids and age-related hearing loss in older adults, *J. Nutr. Health Aging* 14 (5) (2010) 347–351, <https://doi.org/10.1007/s12603-010-0078-x>.
- [114] S.G. Curhan, R.D. Eavey, M. Wang, E.B. Rimm, G.C. Curhan, Fish and Fatty Acid Consumption and the Risk of Hearing Loss in Women 1 – 3 vol. 6, (2014), pp. 1371–1377, <https://doi.org/10.3945/ajcn.114.091819>. Acquired.
- [115] R. Rodrigo, P. Korantzopoulos, M. Cereceda, et al., A randomized controlled trial to prevent post-operative atrial fibrillation by antioxidant reinforcement, *J. Am. Coll. Cardiol.* 62 (16) (2013) 1457–1465, <https://doi.org/10.1016/j.jacc.2013.07.014>.
- [116] M. Sorice, F.P. Tritto, C. Sordelli, R. Gregorio, L. Piazza, N-3 polyunsaturated fatty acids reduces post-operative atrial fibrillation incidence in patients undergoing “on-pump” coronary artery bypass graft surgery, *Monaldi Arch chest Dis = Arch Monaldi per le Mal del torace* 76 (2) (2011) 93–98, <https://doi.org/10.4081/monaldi.2011.196>.
- [117] P. Torre, K.J. Cruickshanks, B.E.K. Klein, R. Klein, D.M. Nondahl, The association between cardiovascular disease and cochlear function in older adults, *J. Speech Lang. Hear. Res.* 48 (2) (2005) 473–481, [https://doi.org/10.1044/1092-4388\(2005\)032](https://doi.org/10.1044/1092-4388(2005)032).
- [118] R. Martínez-Vega, T. Partearroyo, N. Vallecillo, G. Varela-Moreiras, M.A. Pajares, I. Varela-Nieto, Long-term omega-3 fatty acid supplementation prevents expression changes in cochlear homocysteine metabolism and ameliorates progressive hearing loss in C57BL/6J mice, *J. Nutr. Biochem.* 26 (12) (2015) 1424–1433, <https://doi.org/10.1016/j.jnutbio.2015.07.011>.
- [119] P. Bougnoux, N. Hajjaji, M.N. Ferrasson, B. Giraudeau, C. Couet, O. Le Floch, Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: a phase II trial, *Br. J. Canc.* 101 (12) (2009) 1978–1985, <https://doi.org/10.1038/sj.bjc.6605441>.
- [120] Gormaz JG. ISRCTN69560410: cardiac damage prevention in patients undergoing chemotherapy for breast cancer using docosahexaenoic acid (DHA) and carvedilol. doi:10.1186/ISRCTN69560410.
- [121] Z.-Q. Wang, M.-T. Chen, R. Zhang, Y. Zhang, W. Li, Y.-G. Li, Docosahexaenoic acid attenuates doxorubicin-induced cytotoxicity and inflammation by suppressing NF- κ B/iNOS/NO signaling pathway activation in H9C2 cardiac cells, *J. Cardiovasc. Pharmacol.* 67 (4) (2016) 283–289, <https://doi.org/10.1097/FJC.0000000000000350>.
- [122] J. Schjøtt, H. Brurok, P. Jynge, K.S. Bjerve, Effects of eicosapentaenoic acid and docosahexaenoic acid diet supplement on tolerance to the cardiotoxicity of epirubicin and to ischaemia reperfusion in the isolated rat heart, *Pharmacol. Toxicol.* 79 (2) (1996) 65–72 <http://www.ncbi.nlm.nih.gov/pubmed/8878248>.
- [123] R. Uygur, C. Aktas, F. Tulubas, S. Alpsay, B. Topcu, O.A. Ozen, Cardioprotective effects of fish omega-3 fatty acids on doxorubicin-induced cardiotoxicity in rats, *Hum. Exp. Toxicol.* 33 (4) (2014) 435–445, <https://doi.org/10.1177/0960327113493304>.
- [124] H.-C. Hsu, C.-Y. Chen, M.-F. Chen, N-3 polyunsaturated fatty acids decrease levels of doxorubicin-induced reactive oxygen species in cardiomyocytes – involvement of uncoupling protein UCP2, *J. Biomed. Sci.* 21 (2014) 101, <https://doi.org/10.1186/s12929-014-0101-3>.
- [125] V.C. Vaughan, M.-R. Hassing, P.A. Lewandowski, Marine polyunsaturated fatty acids and cancer therapy, *Br. J. Canc.* 108 (3) (2013) 486–492, <https://doi.org/10.1038/bjc.2012.586>.
- [126] P.D. Biondo, D.N. Brindley, M.B. Sawyer, C.J. Field, The potential for treatment with dietary long-chain polyunsaturated n-3 fatty acids during chemotherapy, *J. Nutr. Biochem.* 19 (12) (2008) 787–796, <https://doi.org/10.1016/j.jnutbio.2008.02.003>.
- [127] E.A. Song, H. Kim, Docosahexaenoic acid induces oxidative DNA damage and apoptosis, and enhances the chemosensitivity of cancer cells, *Int. J. Mol. Sci.* 17 (8) (2016) 1257, <https://doi.org/10.3390/ijms17081257>.
- [128] Z. Nikolakopoulou, G. Nteliopoulos, A.T. Michael-Titus, E.K. Parkinson, Omega-3 polyunsaturated fatty acids selectively inhibit growth in neoplastic oral keratinocytes by differentially activating ERK1/2, *Carcinogenesis* 34 (12) (2013) 2716–2725, <https://doi.org/10.1093/carcin/bgt257>.
- [129] M. Moloudizargari, E. Mortaz, M.H. Asghari, I.M. Adcock, F.A. Redegeld, J. Garssen, Effects of the polyunsaturated fatty acids, EPA and DHA, on hematological malignancies: a systematic review, *Oncotarget* 9 (14) (2018) 11858–11875, <https://doi.org/10.18632/oncotarget.24405>.
- [130] O. Zanoaga, A. Jurj, L. Raduly, et al., Implications of dietary ω -3 and ω -6 polyunsaturated fatty acids in breast cancer (Review), *Exp Ther Med* 15 (2) (2017) 1167–1176, <https://doi.org/10.3892/etm.2017.5515>.
- [131] E. Giovannucci, E.B. Rimm, G.A. Colditz, et al., A prospective study of dietary fat and risk of prostate cancer, *J. Natl. Cancer Inst.* 85 (19) (1993) 1571–1579 <http://www.ncbi.nlm.nih.gov/pubmed/8105097>.
- [132] C. Pelsler, A.M. Mondul, A.R. Hollenbeck, Y. Park, Dietary fat, fatty acids, and risk

- of prostate cancer in the NIH-AARP diet and health study, *Cancer Epidemiol. Biomark. Prev.* 22 (4) (2013) 697–707, <https://doi.org/10.1158/1055-9965.EPI-12-1196-T>.
- [133] D. D'Eliseo, F. Velotti, Omega-3 fatty acids and cancer cell cytotoxicity: implications for multi-targeted cancer therapy, *J. Clin. Med.* 5 (2) (2016), <https://doi.org/10.3390/jcm5020015>.
- [134] N. Merendino, L. Costantini, L. Manzi, R. Molinari, D. D'Eliseo, F. Velotti, Dietary ω -3 polyunsaturated fatty acid DHA: a potential adjuvant in the treatment of cancer, *BioMed Res. Int.* 2013 (2013) 310186, <https://doi.org/10.1155/2013/310186>.
- [135] P.A. Corsetto, I. Colombo, J. Kopecka, A.M. Rizzo, C. Riganti, ω -3 long chain polyunsaturated fatty acids as sensitizing agents and multidrug resistance revertants in cancer therapy, *Int. J. Mol. Sci.* 18 (12) (2017) 2770, <https://doi.org/10.3390/ijms18122770>.
- [136] M.H. Raza, S. Siraj, A. Arshad, et al., ROS-modulated therapeutic approaches in cancer treatment, *J. Canc. Res. Clin. Oncol.* 143 (9) (2017) 1789–1809, <https://doi.org/10.1007/s00432-017-2464-9>.
- [137] C. Gorrini, I.S. Harris, T.W. Mak, Modulation of oxidative stress as an anticancer strategy, *Nat. Rev. Drug Discov.* 12 (12) (2013) 931–947, <https://doi.org/10.1038/nrd4002>.
- [138] C. Hegedűs, K. Kovács, Z. Polgár, et al., Redox control of cancer cell destruction, *Redox Biol* 16 (2018) 59–74, <https://doi.org/10.1016/j.redox.2018.01.015>.
- [139] J.N. Moloney, T.G. Cotter, ROS signalling in the biology of cancer, *Semin. Cell Dev. Biol.* 80 (2018) 50–64, <https://doi.org/10.1016/j.semcdb.2017.05.023>.
- [140] L. Tong, C.-C. Chuang, S. Wu, L. Zuo, Reactive oxygen species in redox cancer therapy, *Canc. Lett.* 367 (1) (2015) 18–25, <https://doi.org/10.1016/j.canlet.2015.07.008>.
- [141] T.P. Szatrowski, C.F. Nathan, Production of large amounts of hydrogen peroxide by human tumor cells, *Canc. Res.* 51 (3) (1991) 794–798 <http://www.ncbi.nlm.nih.gov/pubmed/1846317>.
- [142] M.-Y. Liou, P. Storz, Reactive oxygen species in cancer, *Free Radic. Res.* 44 (5) (2010) 479–496, <https://doi.org/10.3109/10715761003667554>. Reactive.
- [143] L. Milkovic, N. Zarkovic, L. Saso, Controversy about pharmacological modulation of Nrf2 for cancer therapy, *Redox Biol* 12 (March) (2017) 727–732, <https://doi.org/10.1016/j.redox.2017.04.013>.
- [144] H.M. Leinonen, E. Kansanen, P. Pölonen, M. Heinänen, A.L. Levonen, Role of the Keap1-Nrf2 Pathway in Cancer vol. 122, (2014), <https://doi.org/10.1016/B978-0-12-420117-0.00008-6>.
- [145] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Mammalian P, exhibit C, metabolism A. Understanding the warburg Effect : cell proliferation, *Science* 324 (May) (2009) 1029, <https://doi.org/10.1126/science.1160809> 80-.
- [146] U.E. Martinez-Outschoorn, M. Peiris-Pagés, R.G. Pestell, F. Sotgia, M.P. Lisanti, Cancer metabolism: a therapeutic perspective, *Nat. Rev. Clin. Oncol.* 14 (2) (2017), <https://doi.org/10.1038/nrclinonc.2017.1> 113-113.
- [147] M.G. Vander Heiden, R.J. DeBerardinis, Understanding the intersections between metabolism and cancer biology, *Cell* 168 (4) (2017) 657–669, <https://doi.org/10.1016/j.cell.2016.12.039>.
- [148] Y. Yang, S. Karakhanova, J. Werner, A. Bazhin, Reactive oxygen species in cancer biology and anticancer therapy, *Curr. Med. Chem.* 20 (30) (2013) 3677–3692, <https://doi.org/10.2174/0929867311320999165>.
- [149] R.A. Siddiqui, K. Harvey, W. Stillwell, Anticancer properties of oxidation products of docosahexaenoic acid, *Chem. Phys. Lipids* 153 (1) (2008) 47–56, <https://doi.org/10.1016/j.chemphyslip.2008.02.009>.
- [150] U.N. Das, M.E. Begin, G. Ells, Y.S. Huang, D.F. Horrobin, Polyunsaturated fatty acids augment free radical generation in tumor cells in vitro, *Biochem. Biophys. Res. Commun.* 145 (1) (1987) 15–24, [https://doi.org/10.1016/0006-291X\(87\)91281-2](https://doi.org/10.1016/0006-291X(87)91281-2).
- [151] A.-M. Engelbrecht, J.-L. du Toit-Kohn, B. Ellis, M. Thomas, T. Nell, R. Smith, Differential induction of apoptosis and inhibition of the PI3-kinase pathway by saturated, monounsaturated and polyunsaturated fatty acids in a colon cancer cell model, *Apoptosis* 13 (11) (2008) 1368–1377, <https://doi.org/10.1007/s10495-008-0260-3>.
- [152] H. Gleissman, R. Yang, K. Martinod, et al., Docosahexaenoic acid metabolome in neural tumors: identification of cytotoxic intermediates, *Faseb. J.* 24 (3) (2010) 906–915, <https://doi.org/10.1096/fj.09-137919>.
- [153] C. Zhang, H. Yu, X. Ni, S. Shen, U.N. Das, Growth inhibitory effect of polyunsaturated fatty acids (PUFAs) on colon cancer cells via their growth inhibitory metabolites and fatty acid composition changes, *PLoS One* 10 (4) (2015) e0123256, <https://doi.org/10.1371/journal.pone.0123256>.
- [154] P.A. Corsetto, A. Cremona, G. Montorfano, et al., Chemical–physical changes in cell membrane microdomains of breast cancer cells after omega-3 PUFA incorporation, *Cell Biochem. Biophys.* 64 (1) (2012) 45–59, <https://doi.org/10.1007/s12013-012-9365-y>.
- [155] M. Murray, A. Hraiki, M. Bebawy, C. Pazderka, T. Rawling, Anti-tumor activities of lipids and lipid analogues and their development as potential anticancer drugs, *Pharmacol. Ther.* 150 (2015) 109–128, <https://doi.org/10.1016/j.pharmthera.2015.01.008>.
- [156] H. Funahashi, M. Satake, S. Hasan, et al., Opposing effects of n-6 and n-3 polyunsaturated fatty acids on pancreatic cancer growth, *Pancreas* 36 (4) (2008) 353–362, <https://doi.org/10.1097/MPA.0b013e31815ccc44>.
- [157] N. Kobayashi, R.J. Barnard, S.M. Henning, et al., Effect of altering dietary -6/-3 fatty acid ratios on prostate cancer membrane composition, cyclooxygenase-2, and prostaglandin E2, *Clin. Canc. Res.* 12 (15) (2006) 4662–4670, <https://doi.org/10.1158/1078-0432.CCR-06-0459>.
- [158] H. Zand, A. Rhimipour, M. Bakhshayesh, M. Shafiee, I. Nour Mohammadi, S. Salimi, Involvement of PPAR-gamma and p53 in DHA-induced apoptosis in Reh cells, *Mol. Cell. Biochem.* 304 (1–2) (2007) 71–77, <https://doi.org/10.1007/s11010-007-9487-5>.
- [159] S.R. Wassall, W. Stillwell, Polyunsaturated fatty acid-cholesterol interactions: domain formation in membranes, *Biochim. Biophys. Acta* 1788 (1) (2009) 24–32, <https://doi.org/10.1016/j.bbmem.2008.10.011>.
- [160] U.N. Das, Essential fatty acids enhance free radical generation and lipid peroxidation to induce apoptosis of tumor cells, *6 (1) (2011) 463–489*, <https://doi.org/10.2217/clp.11.34>.
- [161] U.N. Das, Y.-S. Huang, M.E. Bēgin, G. Ells, D.F. Horrobin, Uptake and distribution of cis-unsaturated fatty acids and their effect on free radical generation in normal and tumor cells in vitro, *Free Radic. Biol. Med.* 3 (1) (1987) 9–14, [https://doi.org/10.1016/0891-5849\(87\)90033-5](https://doi.org/10.1016/0891-5849(87)90033-5).
- [162] R. Gorjao, C.M.M. dos Santos, T.D.A. Serdan, et al., New insights on the regulation of cancer cachexia by N-3 polyunsaturated fatty acids, *Pharmacol. Ther.* 196 (2019) 117–134, <https://doi.org/10.1016/j.pharmthera.2018.12.001>.
- [163] C.A. Gogos, P. Ginopoulos, N.C. Zoumbos, F.K. E Apostolidou, The effect of dietary omega-3 polyunsaturated fatty acids on T-lymphocyte subsets of patients with solid tumors, *Canc. Detect. Prev.* 19 (5) (1995) 415–417.
- [164] S. Gutiérrez, S.L. Svahn, M.E. Johansson, Effects of omega-3 fatty acids on immune cells, *Int. J. Mol. Sci.* 20 (20) (2019), <https://doi.org/10.3390/ijms20205028>.
- [165] S. Khadge, J.G. Sharp, T.R. McGuire, et al., Immune regulation and anti-cancer activity by lipid inflammatory mediators, *Int. Immunopharm.* 65 (2018) 580–592, <https://doi.org/10.1016/j.intimp.2018.10.026>.
- [166] S. Khadge, G.M. Thiele, J.G. Sharp, et al., Long-chain omega-3 polyunsaturated fatty acids decrease mammary tumor growth, multiorgan metastasis and enhance survival, *Clin. Exp. Metastasis* 35 (8) (2018) 797–818, <https://doi.org/10.1007/s10585-018-9941-7>.
- [167] Y. Ye, N.N. Scheff, D. Bernabé, et al., Anti-cancer and analgesic effects of resolvin D2 in oral squamous cell carcinoma, *Neuropharmacology* 139 (2018) 182–193, <https://doi.org/10.1016/j.neuropharm.2018.07.016>.
- [168] L. Sun, Y. Wang, L. Wang, et al., Resolvin D1 prevents epithelial-mesenchymal transition and reduces the stemness features of hepatocellular carcinoma by inhibiting paracrine of cancer-associated fibroblast-derived COMP, *J. Exp. Clin. Canc. Res.* 38 (1) (2019), <https://doi.org/10.1186/s13046-019-1163-6>.
- [169] H.J. Lee, M.K. Park, E.J. Lee, C.H. Lee, Resolvin D1 inhibits TGF- β 1-induced epithelial mesenchymal transition of A549 lung cancer cells via lipoxin A4 receptor/formyl peptide receptor 2 and GPR32, *Int. J. Biochem. Cell Biol.* 45 (12) (2013) 2801–2807, <https://doi.org/10.1016/j.biocel.2013.09.018>.
- [170] U.N. Das, Can bioactive lipid(s) augment anti-cancer action of immunotherapy and prevent cytokine storm? *Arch. Med. Res.* 50 (6) (2019) 342–349, <https://doi.org/10.1016/j.arcmed.2019.10.004>.
- [171] D. Trachootham, J. Alexandre, P. Huang, Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* 8 (7) (2009) 579–591, <https://doi.org/10.1038/nrd2803>.
- [172] A. Kuehne, H. Emmert, J. Soehle, et al., Acute activation of oxidative pentose phosphate pathway as first-line response to oxidative stress in human skin cells, *Mol. Cell* 59 (3) (2015) 359–371, <https://doi.org/10.1016/j.molcel.2015.06.017>.
- [173] S. Hong, Y. Lu, R. Yang, K.H. Gotlinger, N.A. Petasis, C.N. Serhan, Resolvin D1, protecting D1, and related docosahexaenoic acid-derived products: analysis via electrospray/low energy tandem mass spectrometry based on spectra and fragmentation mechanisms, *J. Am. Soc. Mass Spectrom.* 18 (1) (2007) 128–144, <https://doi.org/10.1016/j.jasms.2006.09.002>.
- [174] C.N. Serhan, M. Arita, S. Hong, K. Gotlinger, Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers, *Lipids* 39 (11) (2004) 1125–1132 <http://www.ncbi.nlm.nih.gov/pubmed/15726828>.
- [175] U.N. Das, Essential fatty acids and their metabolites as modulators of stem cell biology with reference to inflammation, cancer, and metastasis, *Canc. Metastasis Rev.* 30 (3–4) (2011) 311–324, <https://doi.org/10.1007/s10555-011-9316-x>.
- [176] Y. Honkura, J. Suzuki, N. Sakayori, et al., Effects of enriched endogenous omega-3 fatty acids on age-related hearing loss in mice, *BMC Res. Notes* 12 (1) (2019), <https://doi.org/10.1186/s13104-019-4809-8>.
- [177] C. Borghi, A. Pirodda, Omega-3 fatty acids: a promising possible treatment for Meniere's Disease and other inner ear disorders of unknown origin? *Med. Hypotheses* 79 (4) (2012) 468–470, <https://doi.org/10.1016/j.mehy.2012.06.024>.
- [178] T. Nakagawa, Roles of prostaglandin E2 in the cochlea, *Hear. Res.* 276 (1–2) (2011) 27–33, <https://doi.org/10.1016/j.heares.2011.01.015>.
- [179] N.G. Bazan, A.E. Musto, E.J. Knott, Endogenous signaling by omega-3 docosahexaenoic acid-derived mediators sustains homeostatic synaptic and circuitry integrity, *Mol. Neurobiol.* 44 (2) (2011) 216–222, <https://doi.org/10.1007/s12035-011-8200-6>.
- [180] R.A. Siddiqui, K.A. Harvey, Z. Xu, E.M. Bammerlin, C. Walker, J.D. Altenburg, Docosahexaenoic acid: a natural powerful adjuvant that improves efficacy for anticancer treatment with no adverse effects, *Biofactors* 37 (6) (2011) 399–412, <https://doi.org/10.1002/biof.181>.
- [181] C.H. Tsai, Y.C. Shen, H.W. Chen, et al., Docosahexaenoic acid increases the expression of oxidative stress-induced growth inhibitor 1 through the PI3K/Akt/Nrf2 signaling pathway in breast cancer cells, *Food Chem. Toxicol.* 108 (Pt A) (2017) 276–288, <https://doi.org/10.1016/j.fct.2017.08.010>.
- [182] F. Picou, C. Debeissat, J. Bourgeois, et al., n-3 Polyunsaturated fatty acids induce acute myeloid leukemia cell death associated with mitochondrial glycolytic switch and Nrf2 pathway activation, *Pharmacol. Res.* 136 (2018) 45–55, <https://doi.org/10.1016/j.phrs.2018.08.015>.