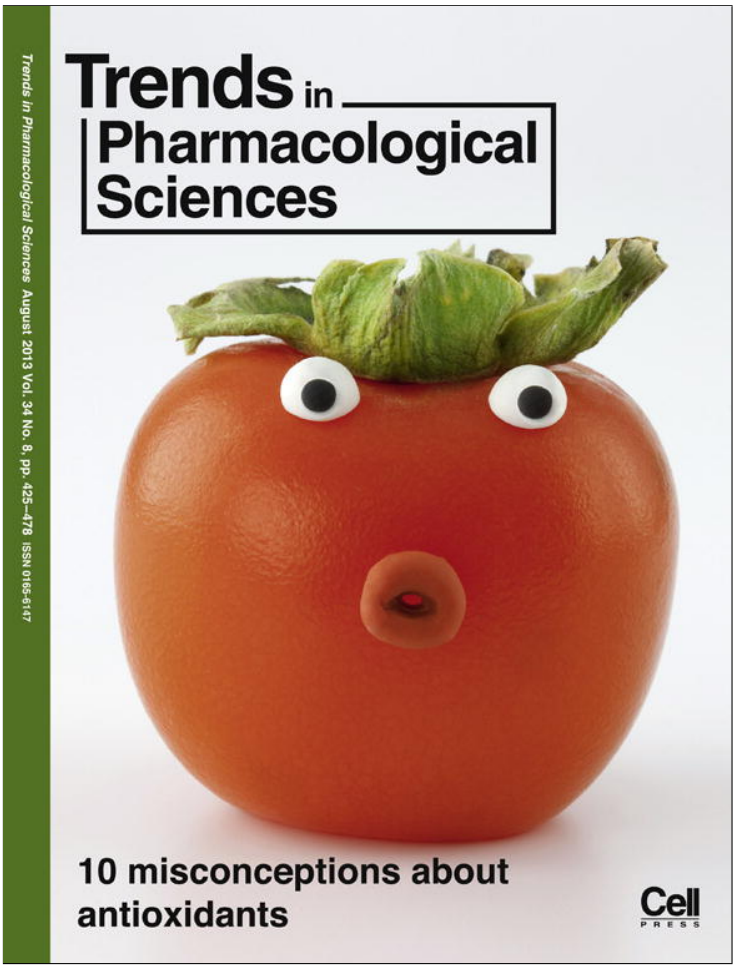

Review

Understanding platinum-induced ototoxicity

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Childhood cancer survival rates are now nearly 80% in more developed European countries because of improved therapies and better supportive care. Platinum chemotherapy drugs, such as cisplatin and carboplatin, are the cornerstone of many effective therapeutic protocols for childhood cancer. However, the antitumor efficacy of cisplatin and carboplatin comes at the cost of ototoxicity, which affects at least 60% of pediatric patients. Although ototoxicity is not life threatening, it can have debilitating effects on patients' quality of life. Recently, many initiatives have been launched with the ultimate goal of reducing cisplatin and high-dose carboplatin ototoxicity without compromising antitumor efficacy. This review addresses the incidence of platinum ototoxicity and its clinical presentation, time course, and early diagnostic evaluation. Genetic and non-genetic risk factors for platinum-associated ototoxicity, and their predictive value, are discussed. Recent developments in the prevention of platinum ototoxicity are also summarized.

Ototoxicity, a platinum-associated side effect, receives renewed attention

The US Food and Drug Administration (FDA) approved cisplatin in 1978 and carboplatin in 1989, despite knowledge of their ototoxic and nephrotoxic side effects. Platinum ototoxicity can manifest in both children and adults. Children, however, are more susceptible to platinum-induced hearing loss than adults, resulting in higher incidence rates (Figure 1). Moreover, deafness due to platinum treatment in childhood is particularly challenging with respect to speech and language development, education, and social integration.

Platinum drugs are essential components in chemotherapeutic regimens for a variety of malignancies, such as osteosarcoma, neuroblastoma, hepatoblastoma, and germ cell tumors in children and metastatic testicular and ovarian tumors, bladder cancer, and non-small cell lung cancer in adults [1]. In countries with a high standard of healthcare, most children diagnosed with cancer in 2013 will be cured. However, many of these children will suffer from disabling ototoxic side effects of platinum chemotherapy. This has catalyzed recent research and initiatives such as the Late Effects Surveillance System and the multidisciplinary European PanCare network have focused on developing strategies for prediction of susceptibility to platinum ototoxicity and early detection and prevention of platinum-induced hearing loss particularly in children. Diagnostic tools for early detection of ototoxicity have been improved, and there has been progress in the identification of pharmacogenetic markers for risk assessment. Moreover, otoprotective compounds against platinum-induced hearing loss are now approaching clinical trials. The objective of this review is to summarize recent evidence obtained from applied/clinical research which has the potential to change care of platinum-treated patients.

Mechanisms

Basic mechanisms of platinum-induced cytotoxicity in normal tissues such as the inner ear are presumably not completely different from those in tumor cells. It is well known that the antineoplastic efficiency of this class of drugs results from the interaction with the nuclear DNA of tumor cells. Platinum compounds such as cisplatin initially induce monoadducts at nucleophilic sites (e.g., of guanine or adenine) and can subsequently lead to intrastrand and interstrand crosslinks in the DNA. Once formed, these lesions can trigger apoptotic cascades predominantly via the mitochondrial pathway. Similar events are observed in cochlear hair cells exposed to platinum drugs [2]. The vital role of DNA adducts in this process is confirmed by the observation that human cells with impaired ability to repair drug-induced damage to their genome through the nucleotide excision repair (NER) pathway are clearly more sensitive to cisplatin than their proficient counterparts.

A factor specifically associated with inner ear damage is drug uptake from stria vascularis into the cochlear fluids and hair cells. Systemic platinum is trafficked across the blood–endolymph barrier and preferentially enter hair cells across their apical membranes [2]. Transport proteins such as megalin (*LRP2*), the organic cation transporter

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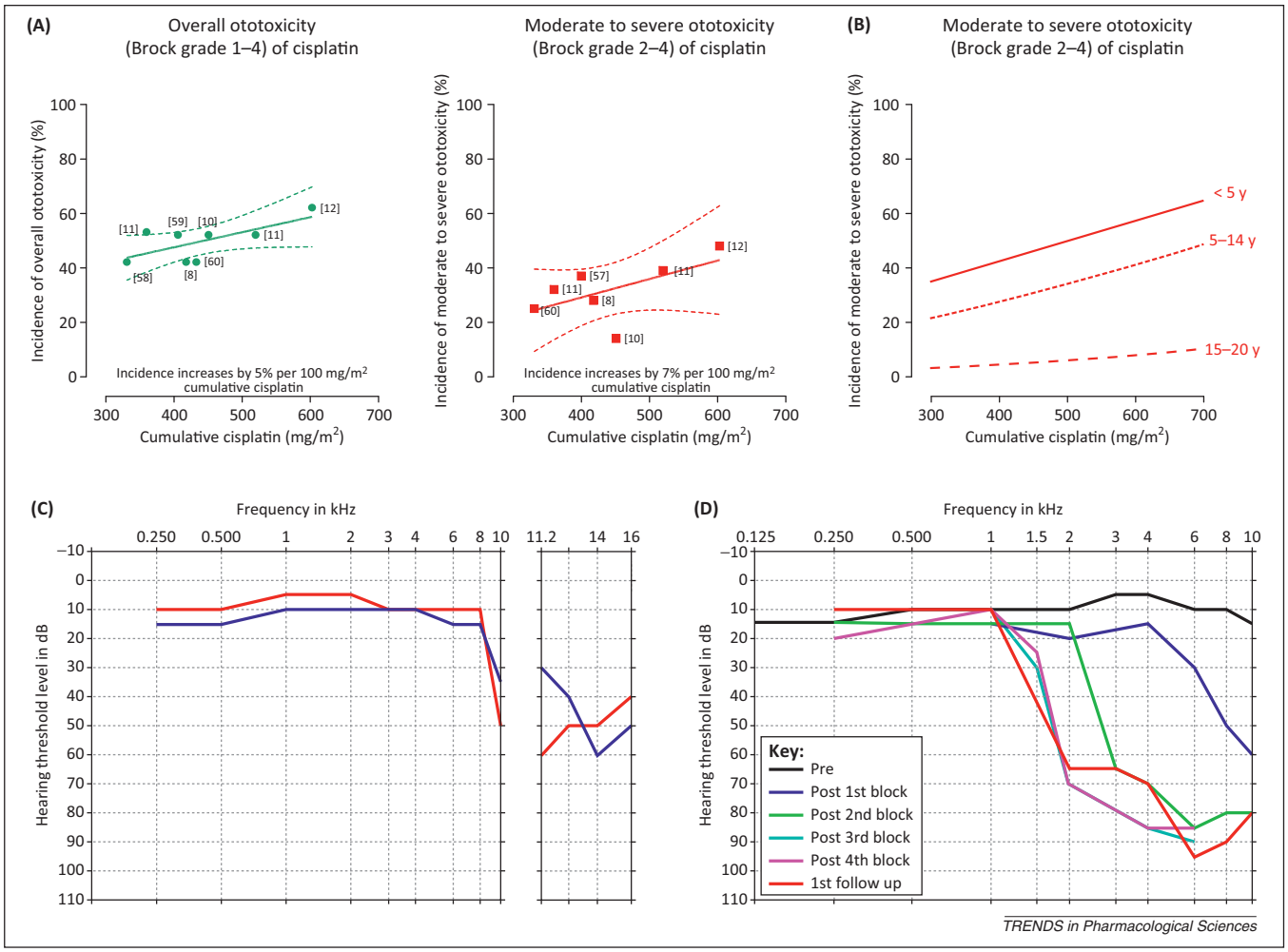

Figure 1. Diagnosis and incidence of cisplatin-associated hearing loss. **(A)** Incidence of ototoxic side effects (overall or moderate to severe toxicity according to Brock *et al.* [12]) following cisplatin treatment in relation to the cumulative dose. Synopsis of published data from pediatric patients (mean age <14 years). The resulting regression lines (solid lines) and the respective 95% confidence intervals (dotted curves) are shown. Data were taken from [8,10–12,59–62]. **(B)** Predicted probability of developing moderate to severe hearing loss as a function of the child's age at treatment and the total cumulative dosage of cisplatin. Adapted from [11], reprinted with permission. **(C)** Example of pure tone and high frequency audiometry in a patient with cisplatin-induced hearing loss >8 kHz (blue, left ear; red, right ear). **(D)** Progressive hearing loss during cisplatin therapy. Shown are left ear audiograms of a patient, recorded at baseline, that is, before start of cisplatin therapy (pre), after each of four cisplatin cycles (post-first block, post-second block, post-third block, post-fourth block), and during follow-up (1st follow up).

OCT2 (*SLC22A2*), or the influx copper transporter CTR1 (*SLC31A1*) are suggested to play an important role in this process [3–5].

Platinum ototoxicity was also attributed to free radical-induced cell damage, and antioxidant as well as platinum detoxifying mechanisms may protect from its ototoxicity. Glutathione *S*-transferases (GSTs) are a group of multifunctional enzymes that catalyze the conjugation of glutathione with a variety of electrophilic compounds including cisplatin, thereby being involved in its detoxification. These enzymes also play an important role in free radical scavenging due to their glutathione-dependent peroxidase activities. In summary, genes involved in transport, metabolism, and in DNA repair have been implicated in the regulation of platinum ototoxicity.

Incidence, risk factors, and clinical presentation

Cisplatin, carboplatin, and oxaliplatin are the only FDA-approved platinum compounds. Because they have been used long-term world-wide, most post-marketing surveillance data are available for these drugs. Overall, the data indicate that cisplatin carries a higher risk of hearing impairment than carboplatin. Nevertheless, treatment with carboplatin still carries a significant risk of mild to severe ototoxicity. Young patients and patients receiving high cumulative doses are at greater risk of carboplatin-induced ototoxicity; the incidence of carboplatin-associated hearing loss can reach 20% in these patients [6]. By contrast, ototoxicity of oxaliplatin is rare. The scientific literature contains only few case reports about ototoxic side effects of this second-generation compound, which is indicated only for treatment of advanced colorectal cancer and thus is not as widely used as cisplatin and carboplatin. Because ototoxic effects of oxaliplatin were reported voluntarily from a population of uncertain size, it is not possible to reliably estimate its frequency. Alongside cisplatin and carboplatin, three other very similar drugs have appeared which have been approved for use in specific countries: nedaplatin (Japan), heptaplatin (South Korea), and lobaplatin (China). Phase II clinical trials directly

comparing cisplatin with its second- and third-generation analogs suggest that there is no difference in the ototoxicity between cisplatin and nedaplatin or heptaplatin [7].

The reported rates of cisplatin-induced ototoxicity in children vary greatly, ranging from 13% to 96% [1,2,8–11]. The variation in the reported incidence rates is attributable in part to differences in cisplatin treatment schedules. The dose and frequency of cisplatin administration differ greatly among treatment protocols, resulting in considerable variation in cumulative doses. Moreover, inconsistencies in the grading scales and assessment times used to monitor cisplatin ototoxicity in clinical trials have made comparisons of incidence data difficult. In 1991, Brock *et al*. introduced the first ototoxicity scale that addressed the typical progression of cisplatin-induced hearing loss, which is characterized initially by high-frequency impairments and later by impairments in lower (speech) frequencies [12] (Table 1). On the basis of the Brock classification, the incidence of overall (grades 1–4) and moderate to severe cisplatin ototoxicity (grades 2–4) ranges from 42% to 62% and 14% to 48%, respectively (Figure 1A).

Platinum ototoxicity usually manifests as bilateral, symmetrical, sensorineural hearing loss and is often accompanied by tinnitus and vertigo. Platinum-induced hearing loss initially affects higher frequencies (≥4 kHz, Figure 1B) and can progress to involve speech frequencies (<4 kHz, Figure 1C). High-frequency hearing loss renders certain consonants (e.g., sibilant sounds) inaudible and may compromise speech recognition and comprehension in young children. It also impairs perception of music and ambient noises (e.g., bird song), resulting in poorer quality of life [13]. Speech perception in background noise is hindered causing a higher perceptual effort at school and poor school performance, particularly in foreign language learning [14,15]. Given that hearing is an integral component of speech development, young children receiving platinum compounds may be at risk for neurocognitive and psychosocial delays [8]. Even if the bilateral hearing loss is mild, children suffer from poor reading skills, word analysis, spelling, phonological short-term memory, and phonological discrimination ability [16].

In some cases, hearing loss progresses even after completion of platinum therapy [17] and may be explained by the prolonged retention of platinum in the body up to 20 years after administration [18]. In fact, platinum plasma concentrations were up to 1000 times higher in ex-patients than unexposed controls [17,18]. Although slight improvements during follow-up have been observed in some patients, platinum-induced hearing damage tends to be permanent [1]. Ototoxic side effects can prove severe, especially in children. A hearing aid may be necessary in the management of as many as 40% of children with platinum-induced hearing impairment [19,20].

Diagnosis

Structural alterations in the outer hair cells and spinal ganglion neurons of the basal cochlear turn represent the earliest event in cisplatin-induced ototoxicity. These structural alterations are accompanied by reduced auditory sensitivity. High-frequency testing (>6 kHz) permits the early detection of platinum-induced ototoxic damage well before impairments become evident in conventional frequency ranges relevant to everyday life of the child. At present, pure tone audiometry (up to 10 kHz), extended high-frequency (EHF) audiometry (up to 16 kHz; Figure 1B), and distortion product emissions (up to 8 kHz) performed with conventional audiological equipment provide information on auditory function at frequencies above 6 kHz. EHF may be particularly useful as a high-quality method to monitor and diagnose early and asymptomatic signs of ototoxicity in patients receiving cisplatin [10,21]. Beahan *et al*. demonstrated high test–retest reliability of EHF for children aged 7 years or older [22]. Singh Chauhan *et al*. found that EHF detected unilateral hearing loss in 31.1% of patients with ototoxic damage before hearing loss became bilateral [23]. Thus, expanding audiometry into the ultrahigh-frequency range can lead to the early detection of hearing loss in a substantial number of cases that would have otherwise been missed [21,23]. This may enable preventive strategies in these patients, such as the use of less ototoxic platinum compounds or the use of otoprotectants if available in the near future.

To establish an association between the platinum drug and hearing loss, ototoxicity monitoring tests require a baseline evaluation usually within 1 week prior to initial treatment. Follow-up evaluations should be performed 24 h prior to each course of platinum-based chemotherapy so that any temporary threshold shift has had time to recover [Durrant, J.D. *et al.* (2009) American Academy of Audiology position statement and clinical practice guidelines: ototoxicity monitoring (http://www.audiology.org/resources/documentlibrary/Documents/OtoMonGuidelines.pdf); Fausti, S.A. *et al.* (1994) Audiologic management of individuals receiving cochleotoxic drug therapy, American Speech-Language-Hearing Association (http://www.asha.org/policy/GL1994-00003.htm)]. To assess possible long-term residual effects of platinum drugs and to detect any progression in the post-drug follow-up, there is a consensus to reevaluate patients every 6 months for the first 2 years post-treatment and then annually for the next 3 years or longer in cases of progressive hearing loss [24].

Recording the kinetics of early audiometric changes during platinum therapy enables the identification of profiles associated with a higher risk of severe hearing loss and needing hearing aids. A grading system that particularly addresses early alterations in high-frequency thresholds is the Muenster classification. This classification includes subgradings within the major hearing loss categories and takes into account minimal hearing losses and the occurrence of tinnitus [25]. Although the Muenster classification includes elements of the high-frequency classifications of Khan *et al*. [26] and Brock *et al*. [12] (Table 1), it detected hearing loss earlier and mapped the progression of hearing impairment more precisely in a study group of 55 children undergoing cisplatin therapy [25].

The evaluation of the accuracy of five different grading systems of cisplatin-induced hearing loss revealed that the American Speech-Language-Hearing Association (ASHA) and the Common Terminology Criteria for Adverse Events (CTCAE) criteria (Table 1) are not effective in the early identification of patients who are at risk of developing

Table 1. Definitions and criteria of ototoxicity and hearing impairment in children

Grade	Khan *et al.* 1982 [26]	Brock's Ototoxicity Grading System 1991 [12]	WHO Grades of Hearing Impairment, pediatric 1997 [61] Mean auditory threshold at 500, 1000, 2000, and 4000 Hz for the best ear	ASHA Guidelines 1994 (http://www.asha.org/policy/GL1994-00003.htm)	Muenster Classification 2007 [25]	NCI CTCAE v. 4.0, pediatric 2010 [62] Auditory thresholds: hearing loss in at least one ear	Chang Practical Grading System 2010 [63]	SIOP Boston Ototoxicity Scale 2012 [2]
0	No toxicity	<40 dB HL at all frequencies	25 dB or less	(A) 20 dB or greater decrease in pure tone threshold at any test frequency	≤10 dB HL at all frequencies		≤20 dB at 1, 2, and 4 kHz	≤20 dB HL at all frequencies
				(B) 10 dB or greater decrease at two adjacent test frequencies				
				(C) Loss of response at three consecutive test frequencies where responses were previously obtained				
1	Tinnitus	≥40 dB HL at 8 kHz	26–40 dB		>10 and ≤20 dB HL at one or more frequencies, or tinnitus	>20 dB HL at 8 kHz	1a: ≥40 dB at any frequency 6 to 12 kHz 1b: >20 and < 40 dB at 4 kHz	>20 dB HL above 4 kHz
2	>20 dB HL at 4 kHz and above	≥40 dB HL at 4–8 kHz	41–60 dB		>20 dB HL at 4 kHz and above 2a: >20 to ≤40 dB 2b: >40 to ≤60 dB 2c: >60 dB	>20 dB HL at 4 kHz and above	2a: ≥40 dB at 4 kHz and above 2b: >20 dB and <40 dB at any frequency below 4 kHz	>20 dB HL at 4 kHz and above
3	>20 dB HL < 4 kHz	≥40 dB HL at 2–8 kHz	61–80 dB		>20 dB HL at <4 kHz 3a: >20 to ≤40 dB 3b: >40 to ≤60 dB 3c: >60 dB	>20 dB HL at 3 kHz and above. Hearing loss sufficient to indicate therapeutic intervention, including hearing aids; additional speech language-related services indicated.	≥40 dB at 2 or 3 kHz and above	>20 dB HL at 2 kHz and above
4	Deafness	≥40 dB HL at 1–8 kHz	81 dB or greater		≥80 dB at <4 kHz	Audiological indication for cochlear implant and additional speech language-related services indicated.	≥40 dB at 1 kHz and above	>40 dB HL at 2 kHz and above

Abbreviations: ASHA, American Speech-Language-Hearing Association; NCI CTCAE, National Cancer Institute, Common Terminology Criteria for Adverse Events; SIOP, International Society of Pediatric Oncology.

ototoxicity because of a lack of specificity and sensitivity, respectively [27]. By contrast, the Muenster classification has the advantage of identifying subgroups with a risk of severe impairment by detecting early auditory changes at high frequencies above 4000 Hz [27]. The presence of a Muenster grade 1 hearing loss after a second cisplatin course had the highest predictive value for needing a hearing aid (sensitivity, 67%; specificity, 87%; associated likelihood ratio, 5.0) [27]. The Muenster classification was optimized to detect early ototoxicity during treatment, whereas other scales such as the CTCAE and the Boston Ototoxicity Scale were developed primarily as outcome measures to report the incidence and severity of acquired hearing loss in children at the completion of platinum treatment [2]. The World Health Organization (WHO) grading system does not evaluate high-frequency hearing loss.

Non-genetic risk factors

Established non-genetic risk factors that increase the susceptibility to ototoxic side effects and hearing loss associated with platinum compounds are summarized in Box 1 (see also [8]). An important risk factor is the dose. At a population level, higher cumulative doses of cisplatin are associated with higher rates of ototoxic side effects; the incidence of cisplatin-dependent ototoxicity increases by an average of 5–7% per additional 100 mg/m^2 cumulative cisplatin (Figure 1A).

Although clinical risk factors are important predictors, they do not fully explain the large interindividual differences in the susceptibility to cisplatin ototoxicity [10,28]. For example, cumulative cisplatin doses of 360–480 mg/m^2 were tolerated without hearing loss in some children with osteosarcoma, whereas other children developed ototoxicity with cumulative doses of only 120 mg/m^2 [29]. Neither total nor free concentrations of platinum in the blood were significant predictors of cisplatin ototoxicity [29], and thus classical therapeutic drug monitoring (TDM) based on blood concentration measurements is not applicable to predict or limit ototoxic side effects of cisplatin. Alternatively, TDM based on *a priori* pharmacogenetic information may be a valid approach and, accordingly, in recent years much effort has been focused on the identification of genetic factors predisposing to platinum ototoxicity.

Box 1. Established clinical risk factors for platinum ototoxicity

- Cotreatment with other potential ototoxic drugs, such as aminoglycoside antibiotics and furosemide.
- Dose and dosing schedule: high risk with high cumulative dose, high dose per course, and bolus application of platinum compounds [8,11]. Within the wide range of cumulative doses used in cancer therapy, a threshold cannot be identified below which cisplatin ototoxicity is absent.
- Cranial irradiation [33].
- Young age at time of exposure: children younger than 5 years have 20-fold increased odds of significant hearing loss than individuals aged 15–20 years [11].
- Sex: males are at moderately (up to 4-fold) greater risk [8].
- Type of platinum compound.

Pharmacogenetics

To date, genetic association studies of ototoxicity have been based on the candidate gene approach. Genes were selected on the basis of established or postulated mechanisms of platinum ototoxicity and a restricted number of polymorphisms in these genes that affect the function or the expression of the encoded protein were evaluated (Figure 2).

The first pharmacogenetic association study was performed by Peters *et al*. more than one decade ago and focused on polymorphisms in genes of the *GST* family [30]. The diverse cisplatin-associated functions of GSTs (i.e., cisplatin detoxification and free radical scavenging) prompted the authors to compare the frequency of reduced-function *GST* polymorphisms in 20 cisplatin ototoxicity cases with that in 19 control patients without hearing impairment. A 3-bp insertion/deletion polymorphism in intron 6 of *GSTM3* (rs1799735), which is known to affect the regulation and ultimately the amount and activity of GSTM3, played a protective role against cisplatin ototoxicity [30].

Oldenburg *et al*. reevaluated potential associations of GST genotypes, namely the c.313A>G (rs1695, p.Ile105Val) single nucleotide polymorphism (SNP) in *GSTP1* and gene deletions in *GSTT1* and *GSTM1*, with cisplatin-induced ototoxicity in 173 testicular cancer survivors. The main result was that presence of both *GSTP1* c.313G minor alleles offered protection against ototoxic side effects of cisplatin [31]. A subsequent screen in 162 pediatric cancer patients, however, failed to replicate the association [32]. A recent study in 69 children who received cisplatin-based chemotherapies and craniospinal radiation for treatment of medulloblastoma showed that carriers of the minor G allele were four times more likely to require hearing aids than non-carriers [33]. Presence of the minor G allele, thus, may increase the risk of radiation-induced but not that of cisplatin-induced hearing loss. This fits well with the literature, which is replete with reports of the *GSTP1* c.313A>G SNP linked with enhanced radiation-associated toxicity [34].

Another candidate gene is *LRP2*, which encodes megalin, a multiligand endocytic receptor abundantly expressed in absorptive epithelia such as renal proximal tubules and epithelia of the inner ear. Megalin has been associated with the uptake of aminoglycosides, which similar to cisplatin have ototoxic and nephrotoxic side effects. Assuming that – by analogy to aminoglycosides – the mechanism of cisplatin-induced ototoxicity involves megalin, Riedemann *et al*. analyzed the frequency of the non-synonymous *LRP2* SNPs rs2075252 and rs4668123 in 50 pediatric cancer patients, a half of those with hearing loss after cisplatin therapy. The authors found a higher frequency of the minor A allele of rs2075252 in the cohort of cases than in controls. The association, however, was not confirmed in subsequent studies [32,35].

Cisplatin causes DNA lesions by forming intrastrand and interstrand crosslinks that result in DNA distortion and inhibition of DNA replication. The NER pathway is one of the major DNA repair systems involved in the removal of platinum adducts. This complex pathway involves the collaboration of many proteins involved in

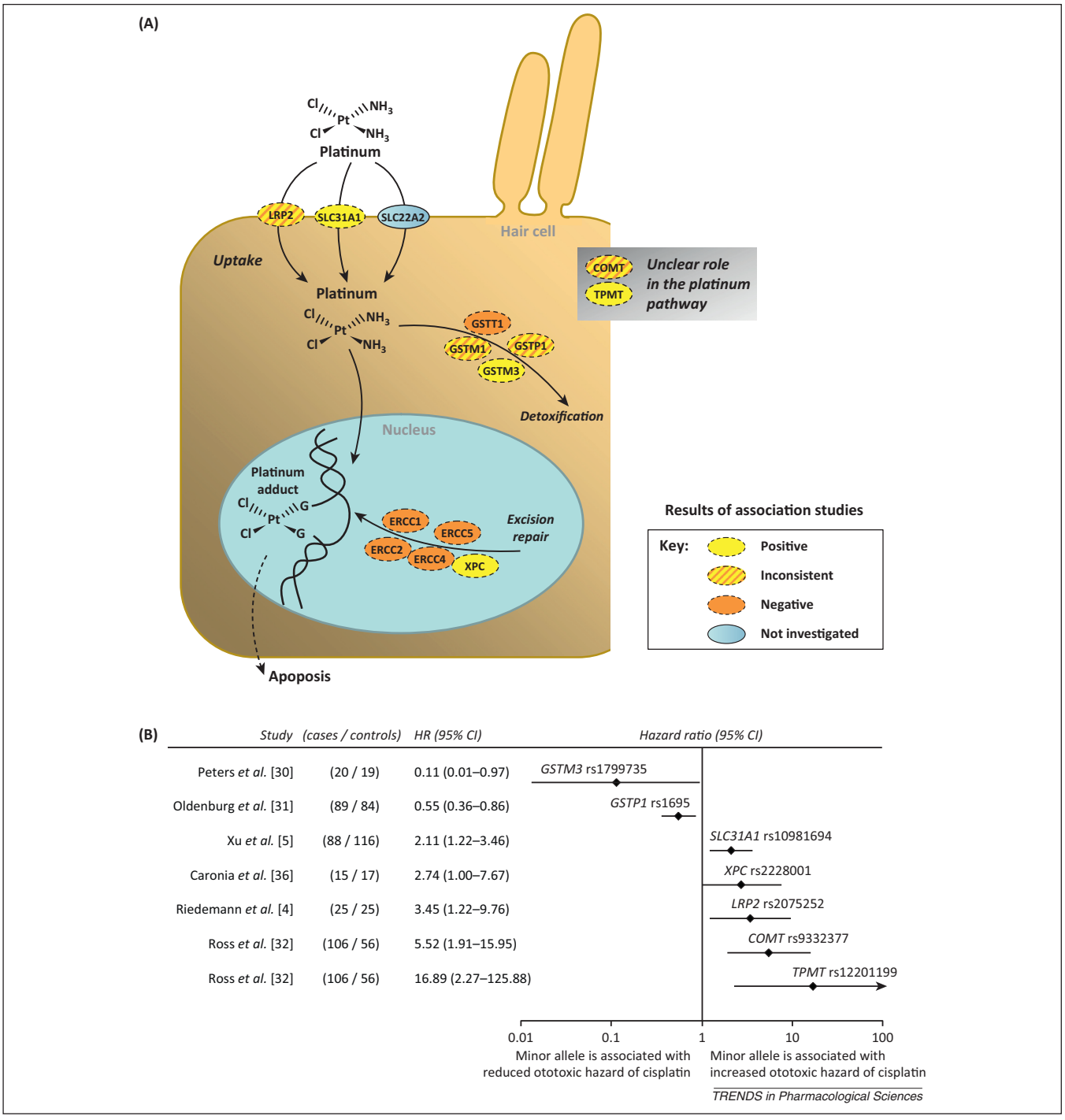

Figure 2. Pharmacogenetic association studies. **(A)** Candidate genes investigated in association studies and their role in the cisplatin pathway. Genes marked with dotted lines have been investigated as candidate genes for cisplatin-induced ototoxicity in pharmacogenetic association studies. **(B)** The Forest plot summarizes studies with significant associations of genetic markers with cisplatin-induced ototoxicity. Analysis by alleles, that is, the total number of wild type and mutant alleles in cases and controls were compared. Filled boxes represent the mean effect size of individual studies with 95% confidence intervals (95% CI) (horizontal lines). Single nucleotide polymorphisms (SNPs) in *GSTM3* (rs1799735) and *TPMT* (rs12201199) show the largest per allele effect. Study references: [4,5,30–32,36]. Abbreviations: *LRP2*, low-density lipoprotein receptor-related protein 2 (megalin); *SLC31A1*, solute carrier family 31 member 1 (copper transporter); *SLC22A2*, solute carrier family 22 member 2 (organic cation transporter 2); *GST*, glutathione *S*-transferase; *ERCC*, excision repair cross-complementing rodent repair deficiency; *XPC*, xeroderma pigmentosum complementation group C; *COMT*, catechol-*O*-methyltransferase; *TPMT*, thiopurine *S*-methyltransferase.

lesion recognition, excision, DNA resynthesis, and ligation. Preclinical and clinical evidence supports a role of the NER pathway in platinum resistance. Although primarily investigating whether polymorphisms in the NER genes *ERCC1*, *ERCC2*, *ERCC4*, *ERCC5*, *XPA*, and *XPC* were associated with tumor response and survival of osteosarcoma patients treated with cisplatin, Caronia *et al*. also addressed the question whether these polymorphisms were predictive of cisplatin ototoxicity in a subcohort of 32 patients [36]. An association of the minor C allele of *XPC* rs2228001 with ototoxic side effects of cisplatin was observed at borderline significance in this explorative hypothesis-generating study.

Two studies investigated whether polymorphisms in genes associated with hereditary deafness segregate more commonly in patients who develop cisplatin ototoxicity. Common causes of non-syndromic deafness are mutations in *GJB2* (connexin 26) and *SLC26A4* (pendrin). In addition to polymorphisms in these nuclear genes, mutations in mitochondrial DNA, particularly in the mitochondrial 12S rRNA and tRNA genes, are also an important cause of non-syndromic sensorineural hearing loss [37]. Interestingly, the m.1555A>G SNP in the 12S rRNA (*RNR1* rs267606617) is predisposing for drug- (aminoglycoside-)induced hearing loss. Assuming that mitochondrial DNA mutations might also predispose for cisplatin-induced hearing loss and thus are more common in patients with cisplatin ototoxicity, Peters *et al*. genotyped 39 pediatric cancer patients with and without cisplatin-related hearing loss. None of the seven tested deafness associated mitochondrial DNA mutations were identified in any of these patients [38]. In a subsequent study, Knoll *et al*. investigated deafness associated mitochondrial mutations (m.1555A>G, m.2343A>G, m.7445A>G) or variants in the 'hearing genes' *GJB2* and *SLC26A4* in 11 cancer survivors with severe cisplatin-induced hearing loss. The frequency of these variants did not significantly deviate from that in the general population [39]. Subject to verification in larger cohorts, these explorative studies did not provide evidence that polymorphisms in genes associated with hereditary deafness segregate more commonly in patients with severe cisplatin ototoxicity and thus might be used as risk markers.

Studies in mice suggest that the influx copper transporter mCtr1 (Slc31a1) is involved in cochlear uptake and toxicity of cisplatin. Transporter expression and substrate specificities can substantially differ between species. In view of the current lack of direct evidence from human studies, it is unclear whether the results derived from animal experiments also hold true for humans. The experimental findings prompted Xu *et al*. to investigate whether *SLC31A1* polymorphisms were associated with cisplatin toxicity in 204 Chinese non-small cell lung cancer patients [5]. Among the 20 tested variants in *SLC31A1*, the rs10981694 tag SNP (i.e., SNP used to 'tag' a particular haplotype) was significantly associated with severe ototoxicity [5]. The study provides first indirect evidence that CTR1/*SLC31A1* could play a role in cisplatin ototoxicity in humans. A weakness of the study is that the causal variant in *SLC31A1* is unknown.

Overall, results from genetic association studies based on the candidate gene approach are mixed; positive associations were initially reported but not replicated in subsequent studies (Figure 2). Replication failure may be due to small sample size, poor case–control definition, inadequate phenotyping, different ancestries of patients, or selection of the wrong candidate gene. Indeed, our knowledge of the mechanisms of platinum ototoxicity is still incomplete. Because the selection of candidate genes at this stage is based on *a priori* knowledge, use of the candidate gene approach to conduct genetic association studies on platinum ototoxicity is likely to have limited success.

A step forward in the identification of genes that contribute to susceptibility to platinum-related ototoxicity was made by Ross *et al*. by extending the number of investigated candidate genes and polymorphisms to include almost 2000 SNPs in 220 genes encoding Phase I and Phase II drug metabolism enzymes, drug transporters, drug targets, drug receptors, transcription factors, ion channels, and genes related to the physiological pathway of platinum [32]. This strategy led to the discovery of the association of tag SNPs rs12201199 in the *thiopurine S-methyltransferase* gene (*TPMT*) and rs9332377 in the *catechol-O-methyltransferase* gene (*COMT*) with cisplatin-induced hearing loss in children. The *TPMT* and *COMT* risk alleles conferred odds ratios of 16.9 [95% confidence interval (CI), 2.3–125.9; $P = 0.03$] and 5.5 (95% CI, 1.9–15.9; $P = 0.03$), respectively [32]. Recently, the associations were replicated for genetic variants in *TPMT* (rs12201199, odds ratio 6.1; 95% CI, 1.8–20.9; $P = 0.0013$) [40].

However, several issues limit the significance of the association of SNPs in *TPMT* and *COMT* with cisplatin-induced hearing loss. First, the link between TPMT and COMT methyltransferases and the physiological pathway of cisplatin has not been established. It has been speculated that *S*-adenosyl methionine (SAM), a key regulator of metabolism, proliferation, differentiation, and apoptosis, could be the missing link in this association. SAM, the main methyl donor group in the cell, is required for TPMT and COMT catalyzed reactions. Inhibition of SAM-dependent transmethylation enhanced the toxicity of cisplatin *in vitro* [41]. Whether SAM mediates the association between polymorphisms in *TPMT* and *COMT* and cisplatin ototoxicity is currently unknown. Second, cisplatin-induced ototoxicity was significantly associated with tag SNPs and not with well-known loss-of-function variants in *TPMT* and *COMT*; therefore, the causal variants in *TPMT* and *COMT* have not yet been identified. Third, the *TPMT* (rs12201199) and *COMT* (rs9332377) tag SNPs were associated with cisplatin ototoxicity in a study cohort consisting primarily of patients of European descent. The frequency of the rs12201199 and rs9332377 risk alleles is 10-fold and 2-fold higher in the African population than the European population, respectively, which suggests that a higher frequency of ototoxicity may occur in Africans. Although not systematically investigated, studies with populations of mixed European and African ancestry, however, did not report significant ethnicity-dependent differences in the incidence of cisplatin-induced hearing loss [11,42]. One possible explanation is that tag SNPs rs12201199 and rs9332377 are in linkage disequilibrium (LD) with unidentified causal variants in European Caucasians but not Africans because of ethnic differences in the LD structure. Consequently, the tag SNPs rs12201199 and rs9332377 may be appropriate as predictive diagnostic markers only in European Caucasians. These findings highlight the need to identify the causal variants in *TPMT* and *COMT*.

An unbiased genome-wide association analysis with sufficient statistical power to confirm previous findings and identify novel causal variants in genes not yet implicated in platinum ototoxicity would be valuable to gain further insight into the mechanisms of platinum-induced hearing loss.

Prevention strategies

The use of lower cumulative cisplatin doses or replacing cisplatin with a second- or third-generation analog with a lower ototoxic potential has not been implemented as a preventative strategy for cisplatin-induced ototoxicity in routine clinical practice, because it is unclear whether these alternative treatments would fully retain the antitumor efficacy and thus survival rates of standard cisplatin regimens. Nevertheless, recommendations for dose adjustments or the replacement of cisplatin with carboplatin are part of some treatment protocols for specific malignancies [27].

A promising alternative approach may be the use of protective agents that allow continued use of optimal platinum chemotherapy while reducing the risk of ototoxicity. In fact, many preclinical studies have been performed to identify otoprotective agents [43–47]. Generation of reactive oxygen species (ROS), which interfere with the antioxidant defense system of the organ of Corti and result in damage to hair cells, has been proposed as the primary mechanism of platinum-induced ototoxicity. Thus, antioxidants, ROS scavengers, and anti-inflammatory drugs may represent potential therapeutic options to prevent platinum-associated ototoxicity.

Amifostine was one of the first compounds tested for otoprotection in clinical trials. Amifostine is a prodrug that is dephosphorylated by alkaline phosphatase in tissues to a pharmacologically active free thiol metabolite, WR-1065. WR-1065 binds to and detoxifies reactive metabolites of cisplatin and may also scavenge ROS generated by cisplatin exposure. WR-1065 is thought to protect normal tissues relative to tumor tissues against oxidative damage inflicted by platinum therapies by becoming concentrated at higher levels in normal tissues. The higher concentration of WR-1065 in normal tissue is attributed to the higher alkaline phosphatase activity, higher pH, and vascular permeation of normal tissue than tumor tissue. Amifostine has been on the market since the mid-1990s for the reduction of the cumulative renal toxicity of cisplatin in patients with advanced ovarian cancer. Unlike its nephroprotective effects, randomized clinical trials and meta-analyses did not provide clear-cut evidence to support the efficacy of amifostine to reduce platinum-induced ototoxicity [48–50]. Consistently, current guidelines do not recommend the routine use of amifostine for the prevention of platinum-associated ototoxicity [51].

D-Methionine has been extensively investigated as an otoprotective agent in preclinical studies for more than two decades. These studies have documented the otoprotective action of D-methionine, most likely due to direct and indirect antioxidative mechanisms, in a variety of species, against a variety of ototoxic insults including cisplatin, carboplatin, aminoglycosides, and noise exposure, supporting clinical trials in these areas. Translation into the clinical development stage has recently started with a Phase III clinical trial testing the potential of D-methionine to reduce noise-induced hearing loss (NCT01345474) [52].

Recent clinical research has focused on another potential otoprotective compound, sodium thiosulfate (STS). STS, a reactive thiol agent, is approved for sequential use with sodium nitrite for the treatment of acute cyanide poisoning. STS is believed to provide otoprotection by directly binding and inactivating platinum cytotoxic agents and acting as a free radical scavenger. In proof-of-concept studies, the otoprotective effect of intravenous STS administered 2 or 4 h after intra-arterial carboplatin was evaluated in both adult and pediatric patients with malignant brain tumors [53,54]. STS, irrespective of treatment schedule, reduced carboplatin-induced ototoxicity when compared with a historical comparison group treated without STS. Detailed case reports suggest that STS treatment does not protect the tumor from platinum cytotoxicity [53]. These early results indicate that STS may offer otoprotection to patients treated with platinum compounds, especially high-risk children. Two large randomized controlled multicenter Phase III studies are currently recruiting a total of 250 pediatric cancer patients to define the role of STS in protection against cisplatin ototoxicity.

Because of the limited data from clinical trials, potential interactions of chemoprotectants with chemotherapy efficacy is still a major concern to oncologists. In particular, the use of chemoprotectants in curative regimens is cautiously regarded and is reflected in the recommendation of the FDA that amifostine should not be administered in curative therapeutic settings. Most potential otoprotective drugs including amifostine and STS are administered systemically. This route of administration may increase the likelihood of an interaction between the chemoprotectant and platinum drug in the systemic circulation or tumor tissue, resulting in the attenuation of the antitumor effects of platinum-based chemotherapy. Changing the application route of the otoprotectant from systemic to local (i.e., transtympanic) administration limits its systemic toxicities and may also help to overcome concerns about tumor protection from platinum cytotoxicity. The practicability and efficacy of transtympanic administration of the otoprotectant *N*-acetylcysteine in 20 cisplatin-treated adult cancer patients was evaluated for the first time by Riga *et al*. in a Phase I/II study [55]. Transtympanic injections of 10% *N*-acetylcysteine significantly reduced the frequency and extent of hearing loss but was accompanied by transient acute pain [55]. The transtympanic route of administration is relatively elaborate and time consuming compared with intravenous or subcutaneous administration. Moreover, hearing loss is not the dose-limiting cisplatin toxicity in adults that it is in children. Therefore, the feasibility and effectiveness of transtympanic injections in children remains to be determined.

Although numerous potential protective agents against platinum-associated ototoxicity have been reported in animal studies, only a few clinical trials have been recently completed. To date, the FDA and the European Medicines Agency have not approved any drug for the prevention of platinum-induced hearing loss. However, STS and *N*-acetylcysteine have received FDA orphan status for this indication.

Concluding remarks

Currently available clinical data were used to develop statistical regression models that predict the risk of developing cisplatin ototoxicity by integrating established clinical variables, namely, patient age and cumulative cisplatin

Table 2. Ongoing clinical studies of otoprotectants (registered studies at ClinicalTrials.gov)

Otoprotectant, proposed mechanism of action	NCT number	Title	Recruitment	Population	Estimated enrollment	Treatment protocol	Study designs	Phases
α-Lipoic acid Antioxidant	NCT00477607	Prevention of cisplatin ototoxicity with the antioxidant α-lipoic acid	Completed, no results available	Adult cancer patients treated with cisplatin-based chemotherapy	200	α-Lipoic acid QD beginning 1 week before the start of cisplatin treatment and continuing for up to 1 month after the completion of cisplatin	Randomized, placebo-controlled, double-blind, multicenter	II/III
Ginkgo biloba extract Antioxidant and ROS scavenger	NCT01139281	The protective effect of *Ginkgo biloba* extract on cisplatin-induced ototoxicity in humans beings evaluated by distortion product otoacoustic emissions	Completed, no results available	Adult patients treated with cisplatin-based chemotherapy	15	*Ginkgo biloba* extract (GBE761) 120 mg BID	Randomized, placebo-controlled, double-blind	II
Sodium thiosulfate Binds and inactivates platinum and acts as a free radical scavenger	NCT00716976	A randomized Phase III study of sodium thiosulfate for the prevention of cisplatin-induced ototoxicity in children	Recruiting	Pediatric cancer patients treated with cisplatin-based chemotherapy	135	Sodium thiosulfate IV over 15 min, beginning 6 h after completion of cisplatin	Randomized, open label, multicenter	III
	NCT00652132	A multi-centre open-label randomised Phase III trial of the efficacy of sodium thiosulphate in reducing ototoxicity in patients receiving cisplatin chemotherapy for standard risk hepatoblastoma	Recruiting	Pediatric hepatoblastoma patients treated with cisplatin-based chemotherapy	115	Sodium thiosulfate IV over 15 min, beginning 6 h after completion of cisplatin	Randomized, open label, multicenter	III
Ringer's lactate Prevention of acidosis; lactate is converted to pyruvate with the generation of reduced nicotinamide adenine dinucleotide, one potent endogenous antioxidant and free radical scavenger	NCT01108601	Transtympanic administration of lactate: an innovative otoprotection for patients receiving cisplatin or carboplatin chemotherapy	Recruiting	Patients 15 years and older undergoing platinum-based chemotherapy	20	Ringer's lactate (+0.03% ciprofloxacin), ear drops BID during chemotherapy	Randomized, open label	I/II
	NCT00584155	Evaluation of lactated ringers for protection from cisplatin ototoxicity	Completed, no results available	Adult patients treated with cisplatin		Ringer's lactate (with 0.03% ofloxacin), ear drops, administered at the start time, 30 min after chemotherapy starts and hourly for 4 h	Randomized, placebo-controlled, single-blind	I
Glucocorticosteroids Attenuation of ROS-generated inflammation	NCT01285674	Intratympanic steroid treatment for the prevention of inner ear toxicity associated with systemic treatment with cisplatin	Not yet recruiting	Adult patients treated with cisplatin-based chemotherapy	20	Intratympanic injection of 0.5 ml methylprednisolone 62.5 mg/ml	Open label	
	NCT01372904	Prevention of cisplatin-induced hearing loss by intratympanic dexamethasone treatment	Recruiting	Adult patients treated with cisplatin-based chemotherapy	30	0.7 ml of dexamethasone phosphate 10 mg/ml injected unilaterally to the middle ear	Randomized, open label	IV
ASA Antioxidant; attenuation of ROS-generated inflammation	NCT00578760	Does aspirin have a protective role against chemotherapeutically induced ototoxicity?	Not yet recruiting	Adult patients treated with cisplatin for germ cell, bladder, or head and neck carcinoma	110	325 mg ASA QD for the duration of cisplatin	Randomized, placebo-controlled, double-blind	

Ebselen Shows glutathione peroxidase-like activity and is a synthetic antioxidant	NCT01451853	Safety and efficacy study of SPI-1005 for prevention of chemotherapy induced hearing loss	Not yet recruiting	Adult patients with advanced head and neck cancer or advanced lung cancer treated with cisplatin-based chemotherapy	80	200, 400, or 600 mg ebselen (SPI-1005) BID, 3 days for each cycle of chemotherapy	Randomized, placebo-controlled, double-blind	II
Amifostine The active metabolite detoxifies reactive metabolites of platinum and scavenges free radicals	NCT00003269	A Phase II, open-label, trial evaluating the efficacy of amifostine in patients with cancers receiving outpatient dose-intensive cyclophosphamide, etoposide, and cisplatin (DICEP) chemotherapy	Completed, no results available	Adult cancer patients receiving outpatient dose-intensive cyclophosphamide, etoposide, and cisplatin chemotherapy	20	Amifostine IV over 15 min daily 30 min prior to high-dose chemotherapy	Open label	II

Abbreviations: QD, *quaque die* (every day); BID, *bis in die* (twice a day); IV, intravenous.

dose. Although these models accurately predict the average risk in a cohort of patients at a given age and cumulative cisplatin dose, they do not precisely map the risk in an individual patient [11]. The reason for this is that only a small fraction of the interindividual variation in ototoxicity response to cisplatin can be explained by clinical variables such as age and cumulative dose. The accuracy of these prediction models may be enhanced by integrating genetic markers such as variants in *TPMT* and *COMT*, which together have a positive predictive value of approximately 90% [32]. In view of the growing pharmacogenetic data, information on the association of *TPMT* polymorphisms with cisplatin-induced hearing loss was included in the FDA drug label for cisplatin. Pretherapeutic genetic tests in pediatric cancer patients may improve medical care but may also have an economic impact. Assuming that an alternative medication exists with the same efficacy and cost as cisplatin but without the risk of hearing loss, Dionne *et al*. estimated that genetic testing for *TPMT* variants in patients treated with first-line platinum therapy could potentially avoid an average of $71 168 in healthcare and societal costs (education costs and lost productivity) per tested patient [56].

The pharmaceutical industry has recognized sensorineural hearing loss as a major global health issue of aging populations and thus as a growing market for novel drug therapies that target inner ear protection and regeneration [Roche announces alliance to discover novel treatments for sensorineural hearing loss (http://www.rocheusa.com/portal/usa/press_releases_nutley?siteUuid=re7180004&paf_gear_id=38400020&pageId=re7425113&synergyaction=show&paf_dm=full&nodeId=1415-05f82de912fe11e2bf151d03aefa681a&currentPage=0)]. It is likely that these recent research and development efforts will also inspire the development of otoprotectants for use in platinum-treated patients. The ultimate goal in the development of otoprotectants is to provide effective protection against inner ear injury without undesirable side effects and interference with the antitumor effects of platinum compounds. The increasing number of Phase III clinical studies that have been started during the past few years (summarized in Table 2) may accelerate the transition from bench to bedside and lead to the approval of an otoprotective agent during the next few years. The next step will be to establish tests (pharmacogenetic tests, scoring systems for clinical risk factors, and audiological tests and grading scales) that will enable the early identification of patients at increased risk for platinum-induced hearing loss and therefore those who will benefit from otoprotective strategies.

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