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Predictors of cisplatin-induced ototoxicity and survival in chemoradiation treated head and neck cancer patients

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ABSTRACT

Objectives: Cisplatin-induced ototoxicity is a common permanent consequence of curative chemoradiation for locally advanced head and neck squamous cell carcinoma (HNSCC). Predictors of ototoxicity in HNSCC were examined.

Materials and methods: In this prospective, observational cohort study, 206 adult HNSCC patients underwent audiometric testing at baseline, during and after treatment with cisplatin-based chemoradiation. Ototoxicity was defined as ≥grade 2 audiometric change from baseline (CTCAE v4.02). Relationships between clinical and pharmacogenetic (*TPMT*, *COMT*, *ACYP2*, *CTR1*, *OCT2*, *MATE1*, *ABCC2*, *ABCC3*, and *ABCG2*) covariates and ototoxicity, progression-free (PFS) and overall survival (OS) were assessed by Cox regression.

Results: Weekly cisplatin resulted in lower ototoxicity risk while PFS and OS were similar compared to high dose cisplatin ($P = 0.00035$; HR = 0.18; 95% CI, 0.07–0.46). *COMT* (rs9332377) carriers had higher ototoxicity risk ($P = 0.00556$; HR = 1.72; 95% CI, 1.17–2.52) while *MATE1* (rs2289669) A/A carriers were protected from ototoxicity ($P = 0.01062$; HR = 0.46; 95% CI, 0.26–0.84). Absence of the protective *MATE1* allele among those who carry the risk allele in *COMT* predicted increased ototoxicity risk, ($P = 0.00414$; HR = 3.22; 95% CI, 1.45–7.17 and $P = 0.00022$; HR = 4.89; 95% CI, 2.11–11.36). Survival outcomes did not differ between carriers of protective or risk alleles.

Conclusions: Weekly cisplatin dosing, *COMT* and *MATE1* are predictors of ototoxicity without affecting treatment efficacy. *COMT* and *MATE1* genotyping and weekly dosing may be a potential strategy for mitigating cisplatin-induced ototoxicity in HNSCC.

Introduction

Cisplatin-based chemoradiation of locally advanced head and neck squamous cell carcinoma (HNSCC) continues to remain the standard treatment regimen despite the high incidence of permanent ototoxicity [1–3]. Over the past 30 years, the incidence of tobacco and alcohol-related HNSCCs has decreased while incidence of human papillomavirus (HPV)-associated oropharyngeal carcinomas has increased leading to a dramatic change in the demographics of patients receiving chemoradiation [4]. With these changes, the largest patient group

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receiving chemoradiation for HNSCC is now a more highly educated, never-smoker, Caucasian male in his 50′s with oropharyngeal cancer [5]. HPV-related oropharyngeal cancers have a greater probability of cure compared to HPV-negative HNSCC (five-year survival rates of > 80% compared to < 50% for HPV-negative status) [6–8]. Consequently, controlling the incidence of permanent treatment-induced toxicities that directly affect functional and occupational status has become an essential priority [4,9].

As HPV-related oropharynx cancer is associated with a better prognosis, de-intensification strategies to maintain cure rates while mitigating adverse effects are being investigated including modifying the use of cisplatin [10,11]. Although weekly cisplatin (40 mg/m^2) appears non-inferior to standard high dose (100 mg/m^2) cisplatin in nasopharyngeal cancer patients, the role of cisplatin schedule on anticancer efficacy and toxicity is less clear in other HNSCC sites [12].

The occurrence of cisplatin-induced ototoxicity is unpredictable even among patients receiving similar treatment regimens suggesting that genetic variation may contribute [2,13]. The onset of this irreversible, bilateral sensorineural hearing loss may also trigger chemotherapy dose modifications that could impact survival benefit. Studies in cisplatin-treated pediatric solid tumor patients have provided conflicting reports suggesting increased risk related to single nucleotide polymorphisms (SNPs) in thiopurine *S*-methyltransferase (*TPMT*), catechol *O*-methyltransferase (*COMT*) and acylphosphatase 2 (*ACYP2*) genes [14–20]. Additional studies have implicated drug transporters involved in cisplatin disposition including copper transporter 1 (*CTR1*), organic cation transporter 2 (OCT2), multidrug and toxin extrusion protein 1 (MATE1) and ATP-binding cassette transporters (*ABCC2, ABCC3* and *ABCG2*) to be associated with platinum response and toxicities [21–25]. Validation of key genetic determinants has been plagued by mixed results. Retrospective study design, varied follow-up periods, inconsistent determination of concurrent radiation, the use of otoprotectant medications, and varying cisplatin treatment protocols may have confounded the effects of genetic associations with cisplatin-induced hearing loss making results difficult to generalize [26,27]. Clinical factors including radiation exposure, cumulative cisplatin dose, and baseline hearing function have been associated with increased risk of ototoxicity [3,9,28].

To our knowledge, there have been no reports investigating genetic predictors of ototoxicity in adult HNSCC patients. Importantly, there is no data to date to support genetic risk alleles identified in pediatric cancer populations are of relevance to this adult disease setting. We prospectively evaluated hearing function in a cohort of HNSCC patients receiving cisplatin-based chemotherapy and intensity modulated radiation therapy (IMRT) to examine the effects of SNPs in candidate genes as well as clinical factors on ototoxicity and survival outcomes.

Methods

Study design and participants

Consecutive consenting adult patients with newly diagnosed locally advanced HNSCC were enrolled in a single-centre, prospective, observational cohort study. All patients were reviewed by a multidisciplinary team and treated with intensity modulated radiation therapy (IMRT) at a dose of 7000 cGy in 35 daily fractions given 5 days per week. Choice of concurrent cisplatin regimen (high dose, 100 mg/m^2 every 3 weeks or weekly, 40 mg/m^2), dose modifications (omission, reductions, switch to cetuximab or carboplatin-5-fluorouracil) and use of alternate radiosensitizers upon toxicity were at the treating physician's discretion. All participants provided written informed consent. This study was approved by the Research Ethics Board at Western University.

Clinical data

Demographic and clinical variables were extracted from electronic medical records. Age was recorded at initiation of cisplatin chemotherapy. Total cochlear radiation dose (Gy) represents the combined left and right mean cochlea dose. Cumulative cisplatin dose (total amount of cisplatin received) and cisplatin dose received until ototoxicity onset was recorded. Routine pathological assessment for HPV was performed in a subset of patients using the surrogate marker p16.

Procedures

Audiology Assessments: Audiometric testing as standard care was performed at baseline, and approximately 21, 42, 90, 182, 365 and 547 days post-cisplatin initiation. Pure tone and speech reception thresholds between 0.25 and 8 kHz were measured bilaterally. Tympanometry and otoacoustic emissions testing were performed. All audiometric data was reviewed blinded to genotype data. Ototoxicity, defined as ≥ grade 2 change from baseline (CTCAE v4.02) with a minimum threshold shift of > 25 dB averaged at 2 contiguous test frequencies in at least one ear was considered non-reversible. An average of the thresholds measured bilaterally from 4 to 8 kHz prior to cisplatin initiation was calculated and used to adjust for baseline hearing function. Hearing protection was recommended to all patients during cisplatin treatment.

Pharmacogenomic testing: We evaluated candidate SNPs previously identified as predictors of ototoxicity in children or adults treated with cisplatin as well as functional SNPs in transporter genes involved in cisplatin disposition. Genomic DNA was extracted from whole blood using the MagNA Pure Compact instrument (Roche, Laval, Quebec, Canada). The following TaqMan allelic discrimination assays (Applied Biosystems, Carlsbad, CA) were used for genotyping: *TPMT* (*A > T*, rs12201199; **2, c.238G > C*, rs1800462; **3B, c.460G > A*, rs1800460; **3C, c.719A > G*, rs1142345; **4, c.626-1G > A*, rs1800584), *COMT* (*C > T*, rs9332377), *ACYP2* (*G > A*, rs1872328), *SLC31A1* (*CTR1 T > G*, rs10981694), *SLC22A2* (*OCT2 c.808G > T*, rs316019), *ABCC2* (*c.-24C > T*, rs717620), *ABCC3* (*c.3890G > A*, rs11568591), *ABCG2* (*c.421C > A*, rs2231142), and *SLC47A1* (*MATE1 G > A*, rs2289669. TPMT variants *2, *3B, *3C and *4 were combined to determine haplotype and associated phenotype, (extensive (EM) or intermediate (IM) metabolizer). Hardy-Weinberg equilibrium was assessed for all genotypes using the Chi square goodness-of-fit test.

Outcomes

The primary objective was to determine covariates associated with time to onset of ototoxicity. Secondary objectives included association of covariates with progression-free survival (PFS) and overall survival (OS). Progression was defined as the date confirming local or distal recurrence. PFS and OS was determined as the date of cisplatin initiation to the date of progression or death, respectively, last contact, or censor date (May 1, 2017), whichever occurred first.

Statistical analysis

Statistical analysis was performed using the statistical software R and GraphPad Prism. Cox proportional hazards model was used to determine significant covariates. Genetic variables were assessed adjusting for age, sex, baseline hearing function, log(cisplatin dose) in a time dependent manner, log(total radiation), and cisplatin regimen. Adjusted *p*-values were obtained using Benjamini-Hochberg procedure to control for false discovery rate for multiple comparisons. Genetic variables with an adjusted *P* value of < 0.2 were considered in the final model. To determine the combined effect of COMT and MATE1, SNPs were stratified in the following groups based on predicted risk: lower risk, *COMT C/C*, *MATE1 A/A*; intermediate risk, *COMT C/C*, *MATE1 G/*

G, *G/A* or *COMT C/T*, *T/T*, *MATE1 A/A*; higher risk, *COMT C/T*, *T/T*, *MATE1 G/G*, *G/A*.

Results

Patient characteristics

223 patients treated between December 2011 and December 2015 consented to participate. Patients with inconclusive audiogram data (n = 11), middle ear dysfunction (n = 1), no available DNA (n = 3), who did not receive cisplatin (n = 1), or were not diagnosed with HNSCC (n = 1) were excluded from analyses. Of the 206 evaluable patients, significant hearing impairment (≥grade 2) following initiation of cisplatin-based chemotherapy developed in 130 (63.1%) patients with an average time to hearing loss of 2.25 months. 182 patients were prescribed high dose cisplatin (100 mg/m^2) every 3 weeks, while 24 patients received weekly (40 mg/m^2) cisplatin. Treatment regimen was significantly associated with ototoxicity ($P < 0.0001$). Median age of patients was lower in those who developed ototoxicity ($P < 0.008$). No differences were noted when comparing patients who developed hearing loss to those who did not for sex, radiation dose, cumulative cisplatin dose, disease site, stage or HPV status (Table 1).

Association between clinical variables and ototoxicity

Clinical and genetic variables were associated with toxicity (Supplemental Table 1) and survival outcomes (Supplemental Tables 2 and 3) using Cox proportional hazards model. Patients receiving weekly cisplatin were significantly protected from developing ototoxicity compared to patients receiving high dose cisplatin ($p < 0.001$) with an adjusted HR of 0.18 (95% CI, 0.07–0.46) (Figs. 1, 2A). While weekly dosed patients were significantly older than patients receiving high dose cisplatin, total cumulative cisplatin dose and total cochlear radiation dose were not different between the two groups (Table 2). PFS and OS were similar between patients treated with high dose and weekly cisplatin (three-year PFS, 81% vs. 70%, $p = 0.7125$; three-year OS, 81% vs. 84%, $p = 0.8526$) (Fig. 2B, C).

At baseline, nearly 70% of patients had normal or mild hearing loss (≤40 dB) between 4 and 8 kHz. An inverse relationship was observed for baseline hearing function as those with normal or mild hearing loss at baseline had higher risk of ototoxicity compared to patients with hearing impairment prior to cisplatin treatment ($p < 0.001$; Supplemental Table 1). A positive association between natural log-transformed total radiation dose and ototoxicity was observed with HR 1.4 (95% CI, 1.07–1.77) (Fig. 1). The effect of natural log-transformed cumulative cisplatin dose (dose received until documented ototoxicity) was determined to be significant in a time-dependent manner ($p < 0.001$). At 12 months post-cisplatin initiation, a trend for a positive association between cumulative cisplatin dose and risk for hearing loss was noted (Fig. 1).

Clinical variables were analyzed in HPV-positive patients with similar results for trends observed for time-dependent cumulative cisplatin dose (Supplemental Table 4). Total radiation exposure and baseline hearing were not significantly associated with onset of ototoxicity in HPV-positive patients. We did not observe an association between weekly cisplatin and ototoxicity among HPV-positive patients potentially due to the small number of patients on this regimen. No clinical or genetic variables tested were associated with PFS and OS in this subset.

Association between genotypes and ototoxicity

Candidate allele frequencies are presented in Table 3. Cox proportional hazards analysis was performed and determined *COMT* (rs9332377) and *MATE1* (rs2289669) SNPs were associated with ototoxicity when adjusted for clinical variables (Table 3). Adjusting for genetic and clinical variables, *COMT* T allele carriers had significantly higher risk of developing ototoxicity (hazard ratio (HR) 1.72, 95% CI 1.17–2.52) while *MATE1* A/A variants were protected from ototoxicity (HR 0.46, 95% CI 0.26–0.84) compared to wild type patients (Supplemental Table 1, Fig. 1).

To assess the combined effect of these SNPs, patients were stratified into putative risk groups based on the presence of protective or risk SNPs in *MATE1* and *COMT*, respectively. Accordingly, 20 (9.7%) patients were categorized as lower risk, 126 (61.2%) at intermediate risk, and 60 (29.1%) at higher risk for ototoxicity. Compared to patients in the lower risk group, intermediate and higher risk patients had significantly higher risk of cisplatin-induced hearing loss with adjusted HRs of 3.22 (95% CI 1.5–7.2) and 4.89 (95% CI 2.1–11.4), respectively (Fig. 3A, Supplemental Table 5). PFS and OS were not different among risk groups (Fig. 3B, C, Supplemental Table 5). Similar results were observed when patients with HPV-related HNSCCs (n = 93) were stratified by risk group (intermediate risk group: HR, 4.85, 95% CI 1.26–18.72; higher risk group: HR, 6.97, 95% CI 1.70–28.64) (Supplemental Table 4). PFS and OS survival rates in HPV-positive patients were high (93% and 90%, respectively) and were not affected by *MATE1* and *COMT* genotypes (Supplemental Fig. 1).

Table 1
HNSCC patient clinical characteristics, n = 206.

	With ototoxicity (n = 130)	Without ototoxicity (n = 76)	P^*
Sex (male)	103 (79.2)	68 (89.5)	0.083
Age at treatment, years			
median (min, max)	56 (31, 78)	59.5 (27, 76)	**0.008**
Self-reported ethnicity			
Caucasian	115 (88.5)	66 (86.8)	0.826
Other[‡]	15 (11.5)	10 (13.2)	
Radiation Dose, median (min, max)			
Total cochlea mean (Gy)	25 (2.2, 102.2)	19 (1.0, 101.3)	0.090
Cisplatin Dose, mg/m^2, median, (min, max)			
cumulative total dose	274.2 (94.8, 511.5)	272.5 (98.6, 527.8)	0.969
Dose received until ototoxicity event or censored	197.2 (39.5, 511.5)	239.2 (39.8, 388.0)	**0.0002**
Treatment regimen			
high dose	124 (95.4)	58 (76.3)	
weekly	6 (4.6)	18 (23.7)	< **0.0001**
Disease site			
oral cavity	15 (11.5)	4 (5.3)	0.211
oropharynx	78 (60)	41 (53.4)	0.465
hypopharynx	5 (3.9)	7 (9.2)	0.131
larynx	10 (7.7)	11 (14.4)	0.153
nasopharynx	10 (7.7)	6 (7.9)	1.000
Primary unknown or preauricular	12 (9.2)	7 (9.2)	1.000
Staging			
I/II	5 (3.9)	5 (6.6)	0.504
III	18 (13.9)	9 (11.8)	0.831
IVA	84 (64.6)	49 (64.4)	1.000
IVB	11 (8.5)	6 (7.9)	1.000
IVC	2 (1.5)	1 (1.3)	1.000
T0	10 (7.7)	6 (7.9)	1.000
HPV Status			
Tested	76 (58.5)	35 (46.1)	0.111
Positive	62 (81.6)	31 (88.6)	
Negative	14 (18.4)	4 (11.4)	0.418

* *P* values were calculated using Fisher's exact test and Mann Whitney test where appropriate.

‡ Other includes African (n = 1), Asian (n = 3), First Nation (n = 8), Middle Eastern (n = 1), and unknown (n = 12) ethnicity.

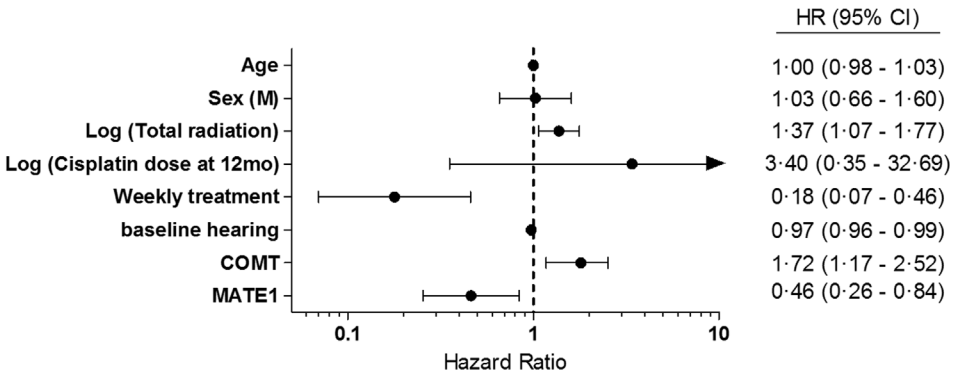

Fig. 1. Hazard ratios of clinical and genetic factors assessed for effect on cisplatin-induced ototoxicity by Cox proportional hazards model. Higher risk of ototoxicity was observed for (Log) total radiation (HR 1.37, 95% CI 1.07–1.77) and *COMT* genotype (HR 1.72, 95% CI 1.17–2.52). Weekly cisplatin dosing (HR 0.18, 95% CI 0.07–0.46) and *MATE1* genotype (HR 0.46, 95% CI 0.26–0.84) were associated with protection from ototoxicity.

Discussion

In this study, over 60% of patients experienced significant hearing loss (≥ grade 2). Weekly administration of cisplatin was associated with lower risk of ototoxicity without compromising treatment efficacy. *MATE1* A/A variants were protected while *COMT* T carriers were at higher risk of hearing loss.

The increasing incidence of HPV-related oropharyngeal cancer has resulted in higher cure rates but places younger patients at lifelong risk for adverse treatment effects. Ototoxicity has a detrimental impact on survivorship quality of life by affecting social and occupational function and has an estimated lifetime economic cost of approximately $300,000 [3]. The ability to identify patients at risk for ototoxicity may contribute to strategies to improve quality of life in cisplatin-treated patients. De-intensification strategies for HNSCC including modifications of concurrent drug therapy with radiation is actively being investigated [10]. We observed that patients treated with weekly cisplatin at 40 mg/m^2 were relatively protected from developing ototoxicity despite receiving similar total cumulative cisplatin doses. Reduced ototoxicity has also been reported with other cisplatin schedules (6 mg/m^2 daily infusions for 20–25 days and 20 mg/m^2 for five days every 4 weeks) compared to standard high dose cisplatin [29,30]. Here, we also determined that PFS and OS did not differ between patients receiving weekly or high dose cisplatin treatments. Recently, Lee et al demonstrated in a randomized phase II study that 3-year progression free survival of nasopharyngeal cancer patients treated with weekly cisplatin was non-inferior to high dose cisplatin treated patients [12]. Together, this data suggests that lower-dose cisplatin regimens may have a significant impact on reducing toxicity while maintaining efficacy in lower risk patients. We were unable to confirm an association between weekly treatment and ototoxicity risk in the subset of HPV-positive patients, likely due to the low number patients receiving weekly cisplatin in this subset. However, our data provides rationale for further studies to examine ototoxicity risk and survival outcomes in HPV-positive patients treated with weekly cisplatin.

To date, pharmacogenomics predictors of cisplatin-induced ototoxicity have been studied primarily in pediatric solid tumor populations. Methyltransferase genes, *TPMT* and *COMT*, are hypothesized to modulate ototoxicity through purine-dependent DNA crosslinking [15,31]. However, conflicting results have been reported for the association between *TPMT, COMT* and ototoxicity, likely due to study design, and confounding variables including concomitant use of ototoxic or otoprotectant medications, radiation, and length of follow-up. *COMT* T allele carriers have been shown to be at increased risk for cisplatin-induced hearing loss in some pediatric studies, while others have failed to observe an effect [14–18]. In our cohort, we note that *COMT* T allele carriers had significantly higher risk of ototoxicity (HR 1.72, 95% CI 1.17–2.52). A recent study of primarily testicular cancer patients also observed a significant association between *COMT* and ototoxicity [32]. Together these data suggest *COMT* may be important across disease settings and an independent predictor of hearing loss regardless of age.

Clinical relevance of *TPMT* genotyping among pediatric patients remains controversial [13–18]. We did not observe an association between *TMPT* rs12201199, **2 *3B*, **3C* or **4* and ototoxicity in adult HNSCC patients further suggesting the role of *TPMT* remains unclear and that a recommendation for *TPMT* pre-emptive pharmacogenetic testing is likely premature. More recently, an association between the acylphosphatase, *ACYP2*, and ototoxicity risk was detected in a genome-wide association study in pediatric patients with brain tumors and further replicated in pediatric osteosarcoma patients [19,20]. Within our adult HNSCC patients, we failed to observe an effect of *ACYP2* variants on hearing loss suggesting disease and age may be important variables.

We further examined the effect of drug transporters on cisplatin-induced ototoxicity. *In vitro* experiments and knock-out studies identified cisplatin as a substrate of MATE1 [33]. We observed MATE1 (rs2289669) homozygous A/A variants were significantly protected from developing ototoxicity (HR 0.46, 95% CI 0.26–0.84). MATE1 is a bidirectional antiporter, expressed on the apical membrane of renal tubular epithelial cells suggesting it may play an important role in modulating clearance and systemic exposure of cisplatin [21,24]. The association of MATE1 (rs2289669) with improved glucose lowering in response to metformin has led to the assumption that this intronic SNP results in reduced MATE1 function within the kidney [34]. However, the bidirectional properties of this antiporter make studying the functional effects of genetic variation *in vitro* under appropriate pH conditions challenging [24]. As we observed a protective effect of MATE1 rs2289669 on cisplatin-induced ototoxicity, the functional effect of this SNP may be substrate dependent leading to differential substrate movement across the brush border membrane domain of tubular epithelial cells. Alternatively, rs2289669 may be in linkage with a gain of function variant allowing A/A patients to exhibit enhanced cisplatin efflux.

Mechanistically, the role of MATE1 in cisplatin-induced toxicities is not well understood. The well-established interplay between the uptake transporter OCT2 and MATE1, is postulated to modulate accumulation of cisplatin within the proximal tubular cells and thereby affect systemic exposure [24,25]. Transporters likely play a role in cisplatin uptake and efflux from cells of the peripheral auditory sensory system as cisplatin can traffic into the inner ear fluid. Cisplatin exposure has been demonstrated to cause outer hair cell death, that is unable to regenerate in mammals leading to permanent hearing loss [3]. Within the cochlea, OCT2 expression has been detected, however, no studies have examined this region for expression of MATE1 [35]. It remains plausible that MATE1 may be expressed locally within the cochlea, with MATE1 A/A variants capable of increased cisplatin efflux. Limited cochlea tissue availability has made studies investigating the role of transporters in ototoxicity challenging. Recently, an improved cisplatin-fluorophore conjugate was developed and may serve as a useful tool for determining the biodistribution of cisplatin in mammalian models [36]. Using this approach in rats, it was evident that the cisplatin-conjugate was able to cross the blood-labyrinth barrier and associated with the

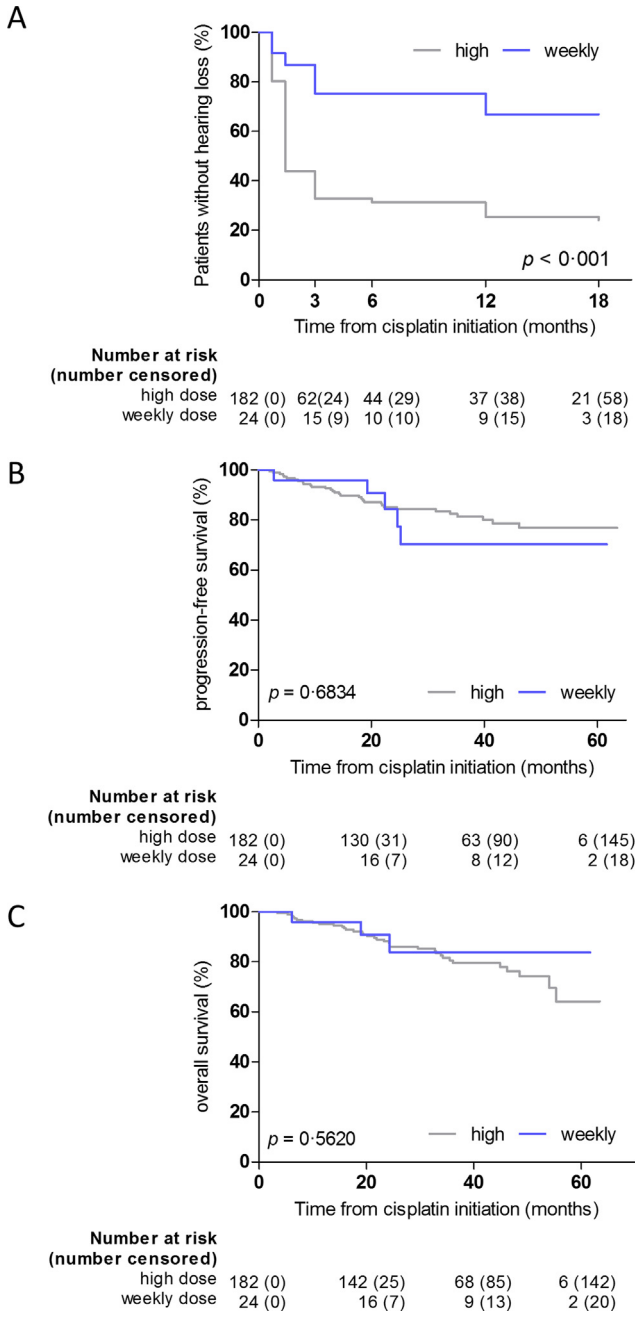

Fig. 2. Kaplan-Meier estimates of (A) onset of cisplatin-induced ototoxicity, (B) progression-free survival, and (C) overall survival for the effect of cisplatin dosing regimen. Patients receiving weekly cisplatin dosing (40 mg/m^2) had significantly reduced ototoxicity compared to patients receiving high dose cisplatin (100 mg/m^2) ($p < 0.001$). No difference in PFS and OS was observed between weekly and high dose cisplatin regimens.

stria vascularis and hair cells within the organ of Corti.

Our data show that patients stratified based on the presence of protective and risk alleles in *MATE1* and *COMT* into intermediate and higher risk groups have higher risk of ototoxicity. PFS and OS was not different between risk groups suggesting that patients identified as lower risk for hearing loss have similar survival outcomes. The effect of our findings for ototoxicity risk was consistent among patients with HPV-related HNSCC. These results suggest that genotyping of *COMT* and *MATE1* may offer some prediction regarding a patient's risk of developing ototoxicity.

Our results from this prospective study suggest that patients with a relatively good cancer prognosis and higher risk of hearing loss may be better suited to a weekly cisplatin dosing regimen. Strategies such as assessing *COMT/MATE1* genotyping and de-intensification of cisplatin therapy using weekly dosing may reduce permanent treatment-related side effects, which is becoming increasingly important in patients with HPV-related carcinomas. However, before recommendations can be made in support of genetic testing prior to cisplatin exposure these results should be validated in a prospective trial designed to investigate the effect of pharmacogenetics and dosing regimen on ototoxicity and survival outcomes in HNSCC.

Table 2
Clinical characteristics of patients prescribed high dose and weekly cisplatin regimens.

	High dose (100 mg/m^2) (n = 182)	Weekly (40 mg/m^2) (n = 24)	P^*
Sex (male), n (%)	149 (81.9)	22 (91.7)	
Age, years, median (min, max)	57 (27,78)	64 (41,76)	0.0040
Ototoxicity, CTCAE score 2–3, n (%)	124 (68.1)	6 (25.0)	< 0.0001
Total cumulative cisplatin dose; mg/m^2, median (min, max)	275.2 (94.8, 527.8)	242.9 (199.0, 327.8)	0.5250
Cumulative dose received until ototoxicity event occurred; mg/m^2, median (min, max)	274.2 (94.8, 511.5)	238.9 (39.5, 282.7)	0.2944
Number of cisplatin cycles received, n (%)			
1	14 (7.7)		
2	45 (24.7)		
3	117 (64.3)		
4 or more	6 (3.3)	24 (100)	
Switched to weekly, n (%)	5 (2.8)	NA	
Total mean radiation dose; Gy, median (min, max)	21.9 (0.96, 102.2)	19.1 (2.1, 77.2)	0.5866
Baseline hearing function, n (%)			
Normal (0–25 dB)	76 (41.8)	4 (16.7)	0.0243
Mild loss (26–40 dB)	59 (32.4)	5 (20.8)	0.3486
Moderate loss (41–55 dB)	30 (16.5)	7 (29.2)	0.1552
Moderately-Severe loss (56–70 dB)	11 (6.0)	6 (25.0)	0.0069
Severe loss (71–90 dB)	6 (3.3)	2 (8.3)	0.2357

* *P* values were calculated using Fisher's exact test and Mann Whitney test where appropriate.

Conflict of interest disclosure statement

The London Regional Cancer Program has received funding from Champions Oncology Inc, Sanofi, Exelixis, Oncogenex, Roche/Genentech, AstraZeneca/MedImmune and Medivation.

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Table 3
Association of genetic variants with cisplatin-induced ototoxicity (n = 206).

		Ototoxicity (n = 206)			
Gene	SNP	n	MAF	*P*	Adj. *P*
TPMT[¥]	EM	188			
	IM	18		0.888	0.888
TPMT	rs12201199				
	A/A	185			
	A/T; T/T	21	0.051	0.353	0.779
COMT	rs9332377				
	C/C	137			
	C/T; T/T	69	0.189	**0.004**	**0.046**
ACYP2	rs1872328				
	G/G	194			
	G/A; A/A	12	0.029	0.345	0.779
CTR1	rs10981694				
	T/T	151			
	T/G; G/G	55	0.136	0.390	0.779
OCT2	rs316019				
	G/G	170			
	G/T; T/T	36	0.097	0.756	0.887
ABCC2	rs717620				
	C/C	137			
	C/T; T/T	69	0.177	0.553	0.829
ABCC3	rs1568591				
	G/G	178			
	G/A; A/A	28	0.070	0.813	0.887
ABCG2	rs2231142				
	C/C	150			
	C/A; A/A	56	0.143	0.534	0.829
MATE1	rs2289669				
	G/G; G/A	177			
	A/A	29	0.386	**0.008**	**0.048**

P values determined by Cox proportional hazards model adjusting for age, sex, time-dependent log(cisplatin dose), log(radiation dose) and treatment schedule. Adj. *P*, adjusted p values determined by Benjamini-Hochberg method.
[¥] TPMT haplotype determined by assessment of rs1800462, rs1800460, rs1142345, rs1800584.

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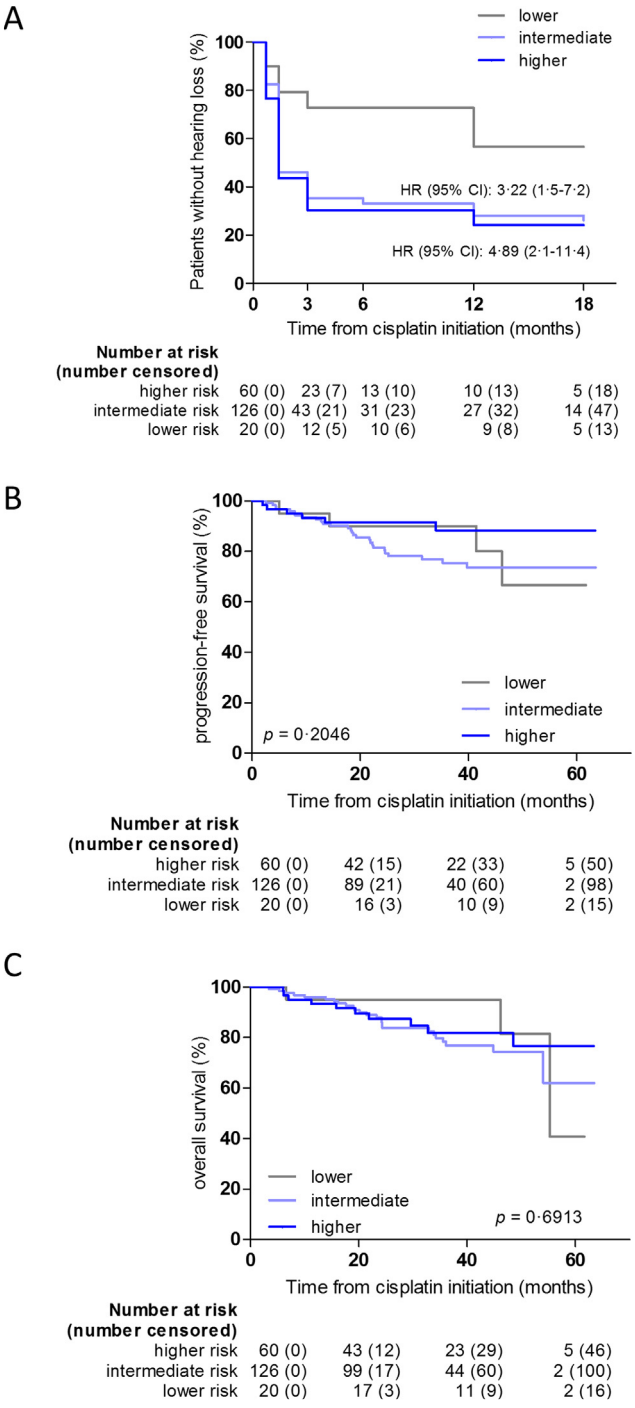

Fig. 3. Kaplan-Meier estimates of onset of (A) onset of cisplatin-induced ototoxicity, (B) progression-free survival, and (C) overall survival for the combined effect of *MATE1* and *COMT* genotypes. The patients were classified into three groups (lower, intermediate or higher risk) based on the presence or absence of protective and risk alleles in *MATE1* and *COMT*, respectively. The intermediate and higher risk groups were at higher risk of ototoxicity compared to the lower risk group. No differences in PFS and OS was observed between risk groups.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2018.12.010.

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