Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fernandez K, Allen P, Campbell M et al. Atorvastatin is associated with cisplatin-induced hearing loss in patients with head and neck cancer.

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List of investigators

Listed in alphabetical order by institution.

Emory University, Department of Otolaryngology – Head and Neck Surgery and Winship Center Institute
- Nicole C. Schmitt

Johns Hopkins University, Department of Radiation Oncology and Molecular Sciences
- Jaylon Garrett
- Brandi Page

Medical University of South Carolina, Department of Otolaryngology – Head and Neck Surgery
- Judy R. Dubno

National Institute on Deafness and Other Communication Disorders, NIH
- Carmen Brewer
- Hui Cheng
- Anna Clements
- Lisa L. Cunningham
- Katharine Fernandez
- Chuan-Ming Li
- Marcia Mulquin
- Nicole C. Schmitt

University of Rochester Medical Center, Department of Medicine
- Deborah Mulford

University of Rochester Medical Center, Department of Otolaryngology
- Paul Allen
- Maura Campbell
- Shawn Newlands

Walter Reed National Military Medical Center
- Thomas Townes
- Candice Ortiz

Inclusion and exclusion criteria

Inclusion criteria

- Newly diagnosed with head and neck squamous cell carcinoma
- Adult, 18 years or older
- Scheduled for treatment with cisplatin

Exclusion criteria

- Prior exposure to cisplatin, taxanes or other cytotoxic chemotherapy drugs
- Baseline audiogram >90 days before onset of first cisplatin treatment
- Baseline hearing ≥95 dB HL average threshold at 1, 2, and 4 kHz
- Indication of active middle ear disease
- Follow up audiogram >90 days after cessation of last cisplatin treatment

Site-specific contributions and study design

This study consisted of combined retrospective and prospective observational data obtained from three clinical sites. Audiometric data collected ≤90 days from the onset of cisplatin therapy were compared against audiometric data collected ≤90 days from completion of cisplatin therapy to determine threshold shifts. Subjects whose baseline audiogram was collected up to 1 week after the first cisplatin infusion (n=12 subjects) were included only if their hearing was within normal limits (≤20 dB HL from 1 to 8 kHz).

Retrospective data were collected from the University of Rochester Medical Center (URMC) (N=215 subjects). All audiometric data were collected in a sound-attenuated booth using either a Grason Stadler GSI 61or SGI AudioStar Pro audiometer and Telephonics TDH50 headphones or EAR ER3A insert earphones and Sennheiser HDA 200 headphones. Air conduction (AC) thresholds for standard frequencies (0.25 to 8 kHz) as well as 12 kHz were obtained. Bone conduction (BC) audiometric thresholds were reported for 1, 2, and 4 kHz and used to screen for the presence of active middle ear disease. Additional retrospective data were collected from Walter Reed National Military Medical Center (WRNMMC) (N=34 subjects). AC thresholds were collected in a sound-attenuated booth for standard audiometric frequencies (0.25 to 8 kHz) as well as over the sensitive range for ototoxicity (SRO), up to 12.5 kHz, using an Otometrics Madsen Astera audiometer and with Sennheiser HDA-200 or RadioEar IP30 headphones. Tympanometry was used to screen for active middle ear disease. Prior to data sharing with NIH collaborators for analyses, URMC and WRNMMC removed personal identifiable information (PII)/personal health information (PHI) from the

dataset. Coded IDs were assigned to each subject, and the code was not shared with NIH investigators.

Prospective data were collected in a collaborative, observational study through a National Institutes of Health (NIH) partnership with Johns Hopkins University (JHU). Audiometric data were collected using an FDA-approved SHOEBOX iPad-based audiometer (Clearwater Clinical, Inc), with Sennheiser HDA-280 headphones (ANSI S3.6),[4] for standard test frequencies (1 to 8 kHz) and extended high frequencies (EHF) including 10 and 12.5 kHz. The SHOEBOX Audiometer has been validated for use outside of a sound booth.[5] All auditory thresholds were measured in a quiet meeting room with SHOEBOX Smart Testing enabled to monitor ambient noise. Tympanometry (MT10 Interacoustics) was used to screen for the presence of active middle ear disease. AC thresholds for all 277 subjects were analyzed at 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12.5 kHz from baseline and post-treatment audiograms. Data from URMC collected at 12 kHz were grouped with 12.5 kHz data from WRNMMC and NIH/JHU. Frequencies at which data were available for <70% of the total number of subjects were excluded from analyses; an example of this is the interoctave frequencies measured using SRO monitoring at WRNMMC only. If a subject had no response at the output limits of the audiometer, a threshold value was assigned as the maximum output level plus 5 dB.

TUNE analysis

Changes in hearing were primarily defined using CTCAEv5.0 criteria.[6] However, cisplatin-induced ototoxicity is characterized initially as a high frequency (above 8 kHz) hearing loss that can spread to include lower frequencies.[5] Therefore, we also applied the TUNE grading scale,[6] which reports incidence and severity of hearing loss based on shifts in auditory thresholds across two frequency ranges: 1 - 4 kHz and 8-12.5 kHz (Table S3). We modified the higher-frequency range of the TUNE scale to include 6, 8 and 12.5 kHz due to insufficient data at 10 kHz in our dataset. Because many of our subjects had some hearing loss at baseline, we further modified the TUNE criteria so that Grades 3 and 4 utilized threshold shift data instead of absolute thresholds. A TUNE Grade 3 was redefined for this study as a ≥ 35 dB PTA threshold shift from the baseline to the post-treatment audiogram, and similarly Grade 4 was redefined as a ≥ 50 dB PTA threshold shift.

Changes in hearing, defined by TUNE criteria, were analyzed using categorical incidence (per ear) data. The incidence and severity distribution of a clinically meaningful hearing change, per ear, relative to statin use was analyzed using chi-square analyses (SAS PROC FREQ procedure). The rate difference, with 95% confidence intervals, of a TUNE-defined hearing loss between atorvastatin and non-statin users was estimated by fitting the Poisson model using PROC NLMIXEDA for the total population as well as for subgroups (sex, cumulative cisplatin dose, individual cisplatin dose, baseline hearing status and radiation). A logistic regression analysis (SAS PROC LOGISTIC procedure) with calculation of odds ratios and 95% confidence

intervals was performed to identify associations between TUNE-defined changes in hearing and statin use after adjustment for significant covariates.

Among subjects not taking any statin, the incidence of a hearing loss per TUNE criteria was 53.4% (Fig. S1A). The incidence of Grade 1 or higher cisplatin-induced hearing loss was significantly reduced relative to the non-statin user group from 53.4% to 39.9% (χ^2= 9.6, p<0.01) in the any-statin user group and 34.0% in the atorvastatin user group, (χ^2= 11.2, p<0.001) (Supplementary Fig. S1A). 36.5% of subjects in the no statin group had a grade 2 or higher change in hearing compared to 14·4% of those in the atorvastatin group (χ^2=21.2, p<0.001) (Fig. S1B)

The logistic regression allowed us to calculate adjusted odds ratios (OR) with 95% confidence intervals for the three variables identified in our mixed effects analysis (MEM) analysis (Table S2) that were significantly associated with cisplatin-induced hearing loss: statin use, cumulative cisplatin dose and baseline hearing status. Using TUNE-defined hearing loss criteria, results indicate that for every 100 mg/m^2 increase in cisplatin dose, a person is 1.8 times more likely to develop hearing loss (OR=1.80, 95% CI:1·36-2.43) (Table S5). Additionally, with every 20 dB increase in PTA threshold at baseline, a person is 44% (OR=0.56, 95% CI: 0.41-0.76) less likely to acquire a cisplatin-induced hearing loss. Finally, an individual on atorvastatin is 56% less likely (OR= 0.44, 95% CI: 0.27-0.72) to acquire a cisplatin-induced hearing loss compared to a non-statin user after controlling for cumulative cisplatin dose and baseline hearing.

Figure S1: Incidence and severity of a TUNE-defined hearing loss in cisplatin-treated patients with head and neck cancer

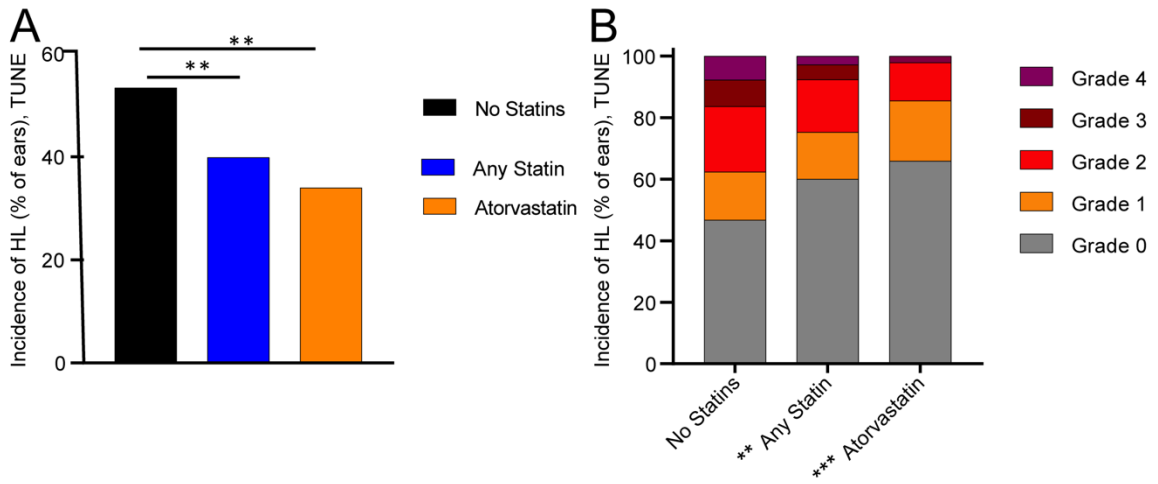

Figure S1: The incidence and severity of cisplatin-induced hearing loss (as defined by TUNE criteria) is reduced among atorvastatin users relative to non-statin users A: The incidence of cisplatin-induced hearing loss is 52% per TUNE criteria amongst non-statin users (black). Subjects taking any statin (blue bar) had significantly lower incidence of cisplatin-induced hearing loss than non-statin users. The incidence of hearing loss was further reduced among atorvastatin users to 34% (orange bar). Data are percent of ears per group. Statistical analysis consisted of Chi-Square, **p<0.01. **B:** Statin use, atorvastatin in particular, is associated with reduced severity of hearing loss. TUNE scale criteria were used to categorize the severity of cisplatin-induced hearing loss. Subjects taking any statin had significantly reduced incidence of a Grade 2 or higher hearing loss compared to non-statin users. This difference was even greater for atorvastatin users. Data are percentage of ears per group. Statistical analysis consisted of Chi-Square, *p<0.05, ***p<0.001.

Figure S2: Correlation of cochlear radiation dose and high frequency hearing sensitivity

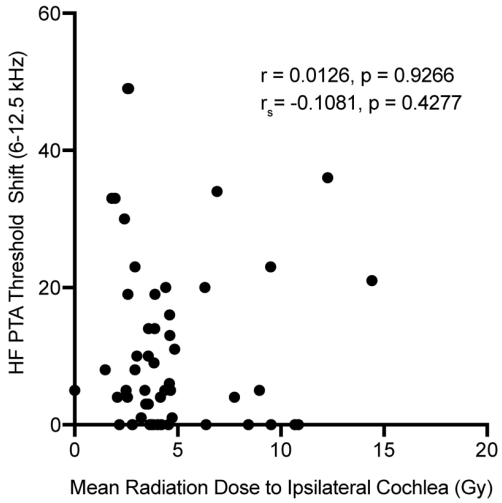

Figure S2: Cochlear radiation dose is not correlated with changes in high frequency hearing sensitivity. Plotted are high frequency (6 to 12.5 kHz) threshold shifts and the mean cochlear radiation dose for each ear in the prospective cohort. Pearson r and Spearman correlation, $p>0.05$. N=56 ears.

Table S1: Study participation criteria

Table S1. Study Participation Criteria	
Inclusion	Exclusion
Adult, 18 yr or older	Prior exposure to cisplatin, taxanes or cytotoxic chemotherapy drugs
Confirmed HNSCC Diagnosis	Profound hearing loss at baseline[A]
Prescribed cisplatin-based chemotherapy	Indication of active middle ear disease[B]

Head and neck squamous cell carcinoma (HNSCC)

[A]Pure tone average (PTA) at 1, 2, and 4 kHz >95 dB hearing level (HL)

[B]Active middle ear disease determined by tympanometry and/or bone conduction audiometry

Table S2: Site contributions

Table S2: Site Contributions			
	Retrospective Data		Prospective Data
	University of Rochester Medical Center (URMC) N=215 (78%)	Walter Reed National Military Medical Center (WRNMMC) N=34 (12%)	National Institutes of Health (NIH)/ Johns Hopkins University (JHU) N=28 (10%)
Subject Characteristics (Table 1)	✓	✓	✓
Concomittent Statin Medications	✓	✓	✓
Cancer Diagnosis/Treatment Parameters	✓	✓	✓
Assessments of Middle Ear Function	Bone Conduction	Tympanometry	Tympanometry
Auditory Assessments	Std.[A] + EHF[B]	SRO[C]	Std.[A] + EHF[B]

[A]Standard audiometric frequencies include 1, 2, 3, 4, 6, and 8 kHz

[B]Extended high frequencies include 10 and 12 or 12.5 kHz

[C]Sensitive region for ototoxicity frequencies include 0.25, 1, 1.5, 2, 3, 4, 5.6, 6, 6.3, 7.1, 8, 9, 10, 11.2, 12.5 kHz

Table S3: Ototoxicity grading criteria

Table S3. Ototoxicity Grading Criteria[A]				
Scale	Frequency Range	Grade	Criteria	Reference
CTCAE	1-8 kHz	Grade 1	Average 15-25 dB TS at 2 consecutive frequencies	National Cancer Institute (NCI), 2017
		Grade 2	Average >25 dB TS at 2 consecutive frequencies	
		Grade 3	Average >25 dB TS at 3 consecutive frequencies	
		Grade 4	Absolute threshold >80 dB at 2 kHz and above	
TUNE	PTA 1-2-4 or 8-10-12 kHz	Grade 1	≥10 dB TS at 1-2-4 kHz or 6-8-12 kHz	Theunissen et al., 2014
		Grade 2	≥20 dB TS at 1-2-4 kHz or 6-8-12 kHz	
		Grade 3[B]	≥35 dB TS at 1-2-4 kHz or 6-8-12 kHz	
		Grade 4[B]	≥50 dB TS at 1-2-4 kHz or 6-8-12 kHz	

[A]Data based on shifts in dB hearing levels obtained using air conduction (AC) audiometry

[B]Scale criteria modified from original reference to accommodate threshold shift data

Table S4: Logistic regression with adjusted odds ratio (OR) on the incidence of a CTCAE or TUNE grade hearing loss

Table S4. Logistic Regression with Adjusted Odds Ratios (OR) on the Incidence of a CTCAE or TUNE Hearing Loss[A]								
	CTCAE				TUNE			
Effect	χ^2	df	*P* value	OR (95% CI)	χ^2	df	*P* value	OR (95% CI)
Atorvastatin Use	7.46	1	0.006	0.48 (0.29 - 0.81)	7.46	1	0.002	0.46 (0.28 - 0.76)
Cisplatin Dose[B]	25.34	1	<0.001	1.01 (1.01 - 1.01)	25.34	1	<0.001	1.01 (1.00 - 1.01)
Baseline Hearing[C]	9.55	1	0.002	0.60 (0.44 - 0.83)	9.55	1	<0.001	0.56 (0.41 - 0.76)

Confidence intervals (CI)

[A]Hearing loss defined as a change in hearing meeting CTCAE or TUNE Grade 1 minimum criteria

[B]Cisplatin dose is cumulative cisplatin dose over length of cisplatin therapy. OR data calculated based on units of 100 mg/m^2

[C]Baseline hearing based on the pure tone average (PTA) of 1, 2, and 4 kHz

Supplementary Appendix References

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