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Efficacy and Safety of AM-111 in the Treatment of Acute Unilateral Sudden Deafness—A Double-blind, Randomized, Placebo-controlled Phase 3 Study

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Objective: To confirm the efficacy and safety of AM-111 (brimapitide), a cell-penetrating c-Jun N-terminal Kinase (JNK) inhibitor, in patients suffering from severe to profound acute unilateral idiopathic sudden sensorineural hearing loss (ISSNHL).

Study design: Prospective, double-blind, randomized, placebo-controlled phase 3 study with follow-up visits on Days 3, 7, 28, and 91.

Setting: Fifty-one European and Asian sites (tertiary referral centers, private ENT practices).

Patients: Two hundred fifty-six patients aged 18 to 65 years presenting within 72 hours following ISSNHL onset with mean hearing loss $\geq$ 40 dB and mean threshold $\geq$ 60 dB at the 3 worst affected contiguous test frequencies.

Interventions: Single-dose intratympanic injection of AM-111 (0.4 or 0.8 mg/ml) or placebo; oral prednisolone as reserve therapy if hearing improvement $<$ 10 dB at Day 7.

Main outcome measures: Hearing improvement to Day 28 was the primary efficacy endpoint; complete hearing recovery, frequency of reserve therapy used, complete tinnitus remission, improvement in word recognition were secondary endpoints. Safety was evaluated by the frequency of clinically relevant hearing deterioration and adverse events.

Results: While the primary efficacy endpoint was not met in the overall study population, post-hoc analysis showed a clinically relevant and nominally significant treatment effect for AM-111 0.4 mg/ml in patients with profound ISSNHL. The study drug and the administration procedure were well tolerated.

Conclusions: AM-111 provides effective otoprotection in case of profound ISSNHL. Activation of the JNK stress kinase, AM-111's pharmacologic target, seems to set in only following pronounced acute cochlear injury associated with large hearing threshold shifts. **Key Words:** AM-111—Apoptosis—Brimapitide—Clinical trial—Hearing loss—ISSNHL—JNK—Peptide—Rescue medication.

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Idiopathic sudden sensorineural hearing loss (ISSNHL) remains one of the most challenging conditions in otology given its acuteness and the prospect of life-long auditory handicap. For patients, the onset of ISSNHL may be a very sudden change if they never experienced hearing problems before (1) and a frightening experience, especially when accompanied by tinnitus and/or vertigo (2). The incidence of ISSNHL has been estimated at 27 per 100,000 in the insured US population

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The trial was registered on the EU Clinical Trials Register (EudraCT 2013-002077-21). It was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation and Good Clinical Practice guidelines. The study was approved by appropriate independent ethics committees and the competent national health authorities.

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(3), 61 per 100,000 for Japan (4), and, more broadly defined, 160 to 265 per 100,000 in Germany (5,6).

Despite extensive research into the pathophysiology of ISSNHL, there still exists no drug treatment that shows unequivocal evidence of efficacy (7). Although frequently used, the therapeutic value of steroids remains unclear—the evidence from randomized controlled trials is contradictory in outcome, in part because the studies are based upon too small a number of patients and also due to methodological shortcomings and reporting issues (8,9). A recent meta-analysis of randomized controlled trials did not support the use of steroids over placebo (10).

AM-111 (brimapitide; Auris Medical AG, Basel, Switzerland) is a 31-amino acid cell-permeable peptide that acts as an inhibitor of the JNK stress kinase. The drug is formulated in a biocompatible hyaluronic acid gel for intratympanic administration after acute hearing loss. The JNK pathway has been well studied in sensory cell apoptosis after mechanical and chemical cochlear stress (11). Various studies have demonstrated the otoprotective potential of AM-111 in a broad range of ototraumatic conditions (12–18) and the importance of the JNK signaling pathway in acute cochlear pathology (19).

The first clinical evidence toward proof of concept for AM-111's otoprotective effects was from a randomized, double-blind, placebo-controlled phase 2 trial, within the first 48 hours following ISSNHL or acute noise trauma (7). Patients suffering from severe-profound hearing loss who were treated with a single dose of AM-111 0.4 mg/ml showed a clinically relevant and nominally significant (without multiplicity adjustment) improvement in hearing and speech discrimination and more frequent tinnitus remission compared with placebo. In patients with mild-moderate hearing loss, no treatment effect was observed due to high rates of spontaneous hearing recovery.

The phase 3 trial HEALOS (Efficacy and Safety of AM-111 in the Treatment of Acute Inner Ear Hearing Loss) was designed to confirm efficacy of AM-111 0.4 mg/ml in the recovery of severe to profound ISSNHL. In addition, HEALOS sought to assess further the dose response relationship and the effect of AM-111 on tinnitus. Acute noise trauma was excluded since such cases had accounted for $< 10\%$ in the previous study.

METHODS

Study Design and Participants

This was a multicenter, double-blind, randomized, placebo-controlled phase 3 trial with three parallel dose groups (AM-111 0.4 mg/ml, 0.8 mg/ml and placebo). The trial involved 51 recruiting sites in 10 European and Asian countries and was registered on the EU Clinical Trials Register (EudraCT 2013-002077-21). It was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation and Good Clinical Practice guidelines. The study was approved by appropriate independent ethics committees and regulatory agencies.

Eligible participants were aged 18 to 65 years, suffered from unilateral ISSNHL with onset up to 72 hours prior, had a hearing threshold $\geq$ 60 dB and mean hearing loss $\geq$ 40 dB at the average of the three worst affected contiguous pure-tone test frequencies (pure-tone average [PTA]). The hearing loss was determined against a reference value: the contralateral ear (20) or, in case of previously asymmetric hearing, a previous audiogram or ISO 7029;2000 norm values. The PTA frequencies determined at baseline remained fixed for all evaluations. Patients presenting within the first 24 hours post ISSNHL had their eligibility reassessed by a confirmatory measure after 24 hours given the substantial spontaneous recovery which occurs early after such incident (7). This confirmatory assessment served as a baseline value.

Exclusion criteria included bilateral ISSNHL, acute hearing loss from noise, baric or head trauma, congenital hearing loss, ISSNHL, autoimmune or radiation-induced hearing loss in the past 2 years, endolymphatic hydrops, Menière's disease, chronic inflammatory or suppurative ear disease, cholesteatoma, acoustic neuroma, otosclerosis, suspected perilymph fistula, or membrane rupture. Patients with active infection of HIV, hepatitis C or B, herpes zoster, otitis media or externa or relevant eardrum abnormality in the affected ear were also excluded. Further, women who were breast feeding, pregnant or who planned a pregnancy during the study, or women of childbearing potential who declared being unwilling or unable to practise an effective method of contraception were not included.

Written informed consent was obtained from each patient before the performance of any study-specific procedures.

Randomization and Masking

At baseline (Day 0), study participants were randomized to receive AM-111 0.4 or 0.8 mg/ml or placebo (vehicle only) at a 1:1:1 ratio. The study drug was identical in appearance for active and placebo doses and revealed no differences during administration. It was provided to study sites in numbered, but otherwise identical kits, each containing one single-dose syringe. Patients were randomized using an interactive web response system with stratification regarding initial PTA frequency range (1, 2, and 3 kHz or lower/2, 3, and 4 kHz or higher), as lower frequency ISSNHL is known to show higher spontaneous recovery (7,21). Patients and investigators remained blinded throughout the entire study.

Procedures

The study consisted of a baseline assessment and four follow-up visits on Days 3, 7, 28, and 91. Baseline safety assessments included a general physical examination, vital signs, hematology and blood chemistry tests and a urine pregnancy test for women of childbearing age. At each study visit, hearing thresholds, word recognition, spontaneous nystagmus, and subjective tinnitus loudness were determined, and the Romberg test was performed.

Hearing thresholds were determined for both ears at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz using the descending method of limits (air and bone conduction). Contralateral masking was required if the interaural difference in air-bone conduction was $>$ 50 dB. Threshold was defined as the lowest audible level, measured twice for that patient. In case of no response due to profound hearing loss, threshold was set at 120 dB. Word recognition score (WRS) was determined for both ears as percentage of correct responses using country-/language-specific word lists at 80 dB stimulus level. At least 20 mono- or disyllabic words were presented in random order. All audiometry output was reviewed by an independent expert.

At baseline, investigators informed patients about the concept of tinnitus and asked them to indicate the presence or absence of tinnitus as well as to rate tinnitus ''at its loudest'' in the past 24 hours on a scale ranging from 0 (''no tinnitus heard'') to 10 (''extremely loud tinnitus''). Subsequently, patients note their tinnitus weekly in a paper or electronic diary.

Approximately 0.25 ml of the study drug was administered on Day 0. For the procedure, the eardrum was anesthetized in accordance with the site's standard practice, the patient was placed in a reclined or supine position with the head tilted 45 degrees toward the unaffected ear, and the drug was applied either through a small tympanotomy or by puncturing the eardrum, preferably in the posterior-inferior quadrant. Patients remained in their position for approximately 30 minutes to allow for drug absorption into the cochlea. Patients whose PTA recovered < 10 dB from baseline to Day 7 were given the option to receive oral prednisolone 50 mg b.i.d. for 4 days followed by a 6-day tapering period.

Endpoints

The primary efficacy endpoint was PTA improvement from baseline to Day 28. Secondary efficacy endpoints included the frequency of patients receiving corticosteroid reserve therapy, complete hearing recovery (PTA recovering to within 10 dB of the reference value) (20), and complete tinnitus remission. The primary safety endpoint was the frequency of clinically relevant hearing deterioration in the treated ear from baseline to Day 28, defined as threshold shift ≥ 10 dB at the average of any two contiguous test frequencies. Secondary safety endpoints included the frequency of clinically relevant hearing deterioration in the treated versus untreated contralateral ear and the frequency and severity of adverse events (AEs) and serious adverse events (SAEs).

Statistical Methods

Efficacy analyses were performed on the ''Intention to Treat'' (ITT) set, which included all randomized patients who were treated with either AM-111 or placebo and had a valid PTA measure at baseline. The ''Safety Population'' analysis set included all patients who received study drug.

For continuous efficacy endpoints, repeated measurement analysis of covariance (ANCOVA) models were used with baseline values of the respective endpoint as covariate and initial PTA frequency range as fixed effect. For binary efficacy endpoints, a logistic regression model was applied, using initial PTA frequency range as covariate. For the primary safety endpoint the Fisher exact test was used, and McNemar's test for the comparison of treated and untreated ears.

The sample size was determined based on the phase 2 trial outcomes. Eighty-five patients per treatment group (i.e., 255 in total) provided about 95% power to detect a treatment effect of 12 dB with a standard deviation (SD) of 20 dB in a 2-sample Z-test at a 4% significance level and 86% power at a 1% significance level (2-sided). The weighted Bonferroni–Holm procedure was applied for multiplicity adjustment, allocating a 4% type 1-error for AM-111 0.4 mg/ml versus placebo and 1% for AM-111 0.8 mg/ml versus placebo.

RESULTS

Patient Flow and Characteristics

The CONSORT trial profile (22) is shown in Figure 1. A total of 258 patients were screened of whom 256 were randomized and treated. A total of 240 patients were included in the ITT set, of whom 95.8% completed the study. Baseline demographics are presented in Table 1 and ear characteristics in Table 2. Mean patient age was 46 years; a slight majority was male (52%). On average, patients were treated 47 hours post ISSNHL onset. The mean baseline PTA was 86.0 dB. In 62% of patients the lower frequencies were most affected; 59% had severe and 41% profound hearing loss (60–89 and $\geq$ 90 dB, respectively) (23). Mean baseline WRS was 34.4%. Tinnitus as comorbidity was present in 84% of patients, whereas vestibular symptoms were rare ($<$4%). Baseline characteristics were similar across treatment groups.

Efficacy Outcomes

Overall, hearing recovery was numerically superior in the AM-111 0.4 mg/ml group compared with placebo at the primary endpoint on Day 28 (38.4 versus 33.8 dB; least square means), however, the primary endpoint was not met ($p = 0.226$). The difference was greater for patients with high frequency PTA; however, the frequency range covariate was not significant ($p = 0.416$). Post-hoc analyses did not show any apparent influence of age, gender, or race; effect size tended to be larger when treated within the first 2 days after onset compared with the third day. However, they revealed a significant difference in the treatment effect for AM-111 0.4 mg/ml as a function of initial hearing loss severity ($p = 0.025$ for the ANCOVA interaction term). Therefore, efficacy endpoints were reassessed separately for the severe and profound ISSNHL subpopulations in further post-hoc analyses.

In patients with profound hearing loss at baseline (Table 3), mean PTA improvement at Day 28 reached 42.7, 37.3, and 26.8 dB in the AM-111 0.4 mg/ml, 0.8 mg/ml and placebo groups, respectively (Table 4, Fig. 2A). The treatment effect for AM-111 0.4 mg/ml reached 15.9 dB and was nominally significant ($p = 0.018$); for AM-111 0.8 mg/ml it was 10.6 dB ($p = 0.126$). The treatment effect was maintained in size to Day 91. In contrast, patients with severe hearing loss at baseline on average showed no separation in PTA improvement between treatment groups (Fig. 2B).

In the profound hearing loss subpopulation, eligibility for reserve therapy was lowest in the AM-111 0.4 mg/ml group (34.3% of patients) and highest in the placebo group (45.5%). Actual use of reserve therapy was different due to the optionality of the treatment as well as protocol deviations. However, within treatment groups the reserve therapy did not seem to influence the course of hearing recovery as the incremental PTA improvement from Day 7 to Day 28 was similar irrespective of whether it was applied or not (Table 5).

The frequency of complete hearing recovery at Day 28 was 18.8%, 17.2%, and 6.5% in the AM-111 0.4 mg/ml, 0.8 mg/ml, and placebo groups, respectively; at Day 91 the frequency reached 21.9%, 20.7%, and 20.0%, respectively. Complete recovery rates were 10 to 20 percentage points higher for patients with low-frequency PTA. Since only a minority of profound ISSNHL

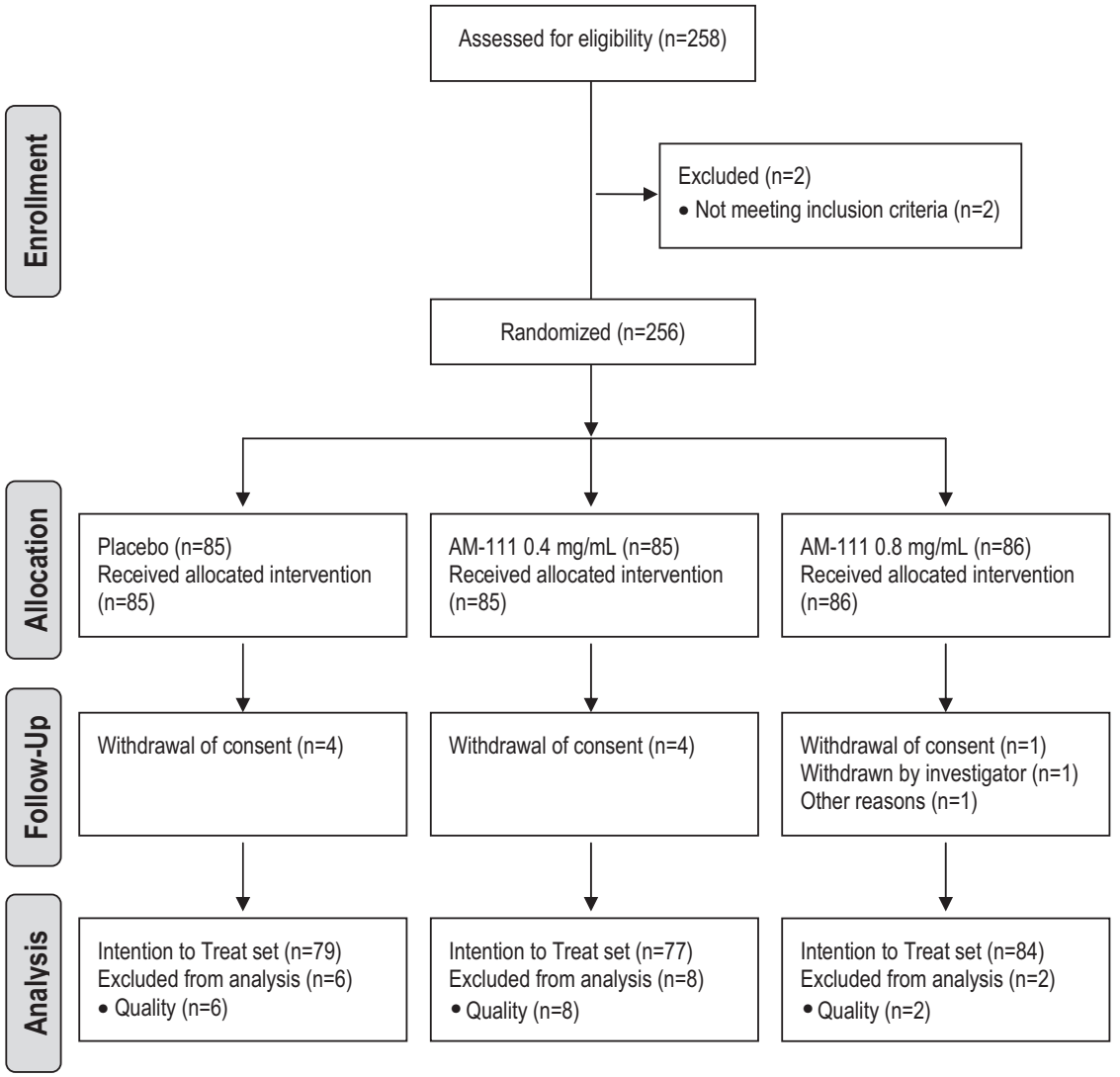

FIG. 1. Patient flow diagram. A total of 258 patients were screened for the HEALOS trial, of whom 256 were enrolled into 1 of 3 parallel treatment groups. Eighty-five patients were randomized into the AM-111 0.4 mg/ml group, 86 into the AM-111 0.8 mg/ml group, and 85 into the placebo group. All patients received 1 single intratympanic administration of study drug and constituted the ''safety population'' analysis set (256 patients). Nine patients withdrew consent, one patient was withdrawn by the investigator, and one was lost to follow-up for other reasons. A total of 240 patients were included in the ''intention-to-treat'' analysis set. Data from 16 patients at 1 site were excluded before database lock due to concerns about the overall quality and reliability of data.

TABLE 1. *Patient demographics at baseline*

	AM-111 0.4 mg/ml N = 85	AM-111 0.8 mg/ml N = 86	Placebo N = 85	Total N = 256
Sex, n (%)				
Male	44 (52)	44 (51)	46 (54)	134 (52)
Female	41 (48)	42 (49)	39 (46)	122 (48)
Age, years				
Mean (SD)	45.8 (13.1)	48.0 (10.9)	45.5 (12.1)	46.4 (12.1)
Range, years	21 to 65	22 to 65	19 to 65	19 to 65
Race, n (%)				
Asian	21 (25)	21 (24)	24 (28)	66 (26)
White/Caucasian	64 (75)	65 (76)	61 (72)	190 (74)

SD indicates standard deviation. ''Safety Population'' analysis set.

patients recovered hearing completely, the relative risk ratio for ''no improvement'' according to Siegel's criteria (< 15 dB) (24) was also evaluated (Table 6). At both Day 28 and Day 91, 11.4% of patients in the AM-111 0.4 mg/ml group showed no improvement, which compares with 41.2% and 38.2%, respectively, in the placebo group; the relative risk ratio versus placebo was < 0.3 ($p = 0.006$ and 0.013, respectively). Results for the AM-111 0.8 mg/ml group were in between, without reaching significance. At the end of the study, 34.3% of patients in the profound hearing loss subpopulation treated with AM-111 0.4 mg/ml had improved to good or serviceable hearing according to the Gardner–Robertson classification compared with 20.6% in the placebo group (Table 7).

TABLE 2. *Baseline ear characteristics entire study population*

	AM-111 0.4 mg/ml N = 77	AM-111 0.8 mg/ml N = 84	Placebo N = 79	Total N = 240
Affected ear, n (%)				
Right	38 (49.4)	43 (51.2)	46 (58.2)	127 (52.9)
Left	39 (50.6)	41 (48.8)	33 (41.8)	113 (47.1)
Time from ISSNHL onset, hours				
Mean (SD)	50.0 (13.0)	44.9 (15.1)	46.4 (14.4)	47.0 (14.3)
Median	51.0	49.1	49.1	49.7
Range	17.8–74.8	10.8–73.6	21.5–74.3	10.8–74.8
PTA of the affected ear at Day 0, dB				
Mean (SD)	88.7 (19.1)	83.3 (18.3)	86.2 (18.3)	86.0 (18.6)
Median	85.0	79.0	85.0	83.0
Range	60 to 120	57 to 120	60 to 120	57 to 120
Initial frequency range, number (%) patients				
Low frequency	50 (64.9)	51 (60.7)	48 (60.8)	149 (62.1)
High frequency	27 (35.1)	33 (39.3)	31 (39.2)	91 (37.9)
Initial severity grade, n (%)				
Severe	42 (54.5)	54 (64.3)	45 (57.0)	141 (58.8)
Profound	35 (45.5)	30 (35.7)	34 (43.0)	99 (41.3)
Word recognition at 80 dB at Day 0, %				
Mean (SD)	33.8 (36.7)	33.6 (33.6)	35.7 (34.6)	34.4 (34.8)
Median	16.0	22.5	30.0	25.0
Range	0 to 100	0 to 100	0 to 100	0 to 100
ISSNHL with tinnitus				
Number (%) patients	64 (83.1)	71 (84.5)	66 (83.5)	201 (83.8)
Tinnitus loudness, mean (SD)	5.7 (2.2)	6.1 (2.5)	6.0 (2.4)	5.9 (2.4)
Vestibular symptoms, n (%)				
Spontaneous nystagmus, abnormal	4 (5.2)	2 (2.4)	3 (3.8)	9 (3.8)
Romberg test positive	3 (3.9)	3 (3.6)	3 (3.8)	9 (3.8)

SD indicates standard deviation; ISSNHL, idiopathic sudden sensorineural hearing loss; PTA, pure-tone average (three most affected contiguous test frequencies); low frequency, mid-point of three worst affected test frequencies at 2 kHz or lower (i.e., PTA frequencies 1, 2, and 3 kHz or lower); high frequency, mid-point above 2 kHz (i.e., PTA frequencies 2, 3, and 4 kHz or higher). ''Intention to treat'' analysis set.

Complete remission of tinnitus at Day 91 was observed in 17.2%, 25.0%, and 19.2% of patients in the AM-111 0.4 mg/ml, 0.8 mg/ml, and placebo groups, respectively; the differences between groups were not significant. The improvement in WRS reached 38.4, 31.0, and 29.2 percentage points at Day 28 and by Day 91 increased further to 49.2, 39.7, and 30.4 percentage points, respectively. The treatment effect for the AM-111 0.4 mg/ml group reached 18.8 percentage points ($p = 0.062$), and for the AM-111 0.8 mg/ml it was 9.4 percentage points ($p = 0.362$).

In a sensitivity analysis with the lower boundary of profound hearing loss set at 80 instead of 90 dB, treatment differences in general became smaller. The difference between AM-111 0.4 mg/ml and placebo reached 9.7 dB ($p = 0.079$) at Day 28, and 11.3 dB at Day 91 ($p = 0.045$). The proportion of patients with no hearing improvement was still nominally significantly lower in the AM-111 0.4 mg/ml group compared to the placebo group at both Day 28 and Day 91 (12.2 vs. 37.8%, $p = 0.007$ and 12.2 vs. 33.3%, $p = 0.024$).

Safety and Tolerability Outcomes

There were no statistically significant differences between treatment groups for the primary safety endpoint: the incidence of clinically relevant hearing deterioration was 5.4%, 2.4%, and 6.6% in the AM-111 0.4 mg/ml, 0.8 mg/ml, and placebo groups, respectively. In the treated ear deterioration was observed more often than in the untreated contralateral ear on Day 3 in the AM-111 0.8 mg/ml and placebo groups ($p < 0.05$), but no more thereafter, suggesting transient effects of the administration procedure.

Treatment-emergent adverse events (TEAEs) were observed for similar proportions of patients across treatment groups with no clinically meaningful differences in frequency, severity, or relationship (Table 8). The majority of TEAEs were local, concerned primarily with hearing and labyrinth disorders, followed in smaller numbers by nervous disorders and infections and infestations.

The majority of TEAEs were mild to moderate in severity. Three patients, all in the placebo group,

TABLE 3. *Baseline hearing characteristics profound hearing loss subgroup*

	AM-111 0.4 mg/ml N = 35	AM-111 0.8 mg/ml N = 30	Placebo N = 34	Total N = 99
Affected ear, n (%)				
Right	15 (42.9)	16 (53.3)	19 (55.9)	50 (50.5)
Left	20 (57.1)	14 (46.7)	15 (44.1)	49 (49.5)
Time from ISSNHL onset, hours				
Mean (SD)	50.0 (13.0)	44.9 (15.1)	46.4 (14.4)	47.0 (14.3)
Median	51.0	49.1	49.1	49.7
Range	17.8–74.8	10.8–73.6	21.5–74.3	10.8–74.8
PTA of the affected ear at Day 0, dB				
Mean (SD)	106.7 (10.8)	104.0 (10.5)	104.1 (11.2)	105.0 (10.8)
Median	97.0	93.0	95.0	95.0
Range	90 to 120	90 to 120	90 to 120	90 to 120
Initial frequency range, number (%) patients				
Low frequency	23 (65.7)	23 (76.7)	20 (58.8)	66 (66.7)
High frequency	12 (34.3)	7 (23.3)	14 (41.2)	33 (33.3)
Word recognition at 80 dB at Day 0, %				
Mean (SD)	9.8 (24.9)	10.5 (21.2)	16.6 (26.5)	12.4 (24.4)
Median	0.0	0.0	0.0	0.0
Range	0 to 100	0 to 80	0 to 85	0 to 100
ISSNHL with tinnitus				
Number (%) patients	32 (91.4)	25 (83.3)	29 (85.3)	86 (86.9)
Tinnitus loudness, mean (SD)	5.6 (2.4)	6.0 (2.4)	5.7 (2.6)	5.8 (2.5)

SD indicates standard deviation; ISSNHL, idiopathic sudden sensorineural hearing loss; PTA, pure-tone average (three most affected contiguous test frequencies): low frequency, mid-point of three worst affected test frequencies at 2 kHz or lower (i.e., PTA frequencies 1, 2, and 3 kHz or lower); high frequency, mid-point above 2 kHz (i.e., PTA frequencies 2, 3, and 4 kHz or higher). ''Intention to treat'' analysis set.

TABLE 4. *Improvement in PTA in profound hearing loss subgroup*

	AM-111 0.4 mg/ml N = 35	AM-111 0.8 mg/ml N = 30	Placebo N = 34
Baseline to Day 3, dB			
Δ PTA LS means (SE)	12.1 (2.6)	11.4 (2.8)	8.8 (2.6)
Δ PTA LS mean difference (SE)	3.3 (3.6)	2.6 (3.7)	
P-value	0.353	0.490	
Baseline to Day 7, dB			
Δ PTA LS means (SE)	21.6 (3.5)	19.5 (3.9)	14.6 (3.6)
Δ PTA LS mean difference (SE)	7.0 (5.0)	4.9 (5.3)	
P-value	0.167	0.349	
Baseline to Day 28, dB			
Δ PTA LS means (SE)	42.7 (4.6)	37.3 (5.0)	26.8 (4.7)
Δ PTA LS mean difference (SE)	15.9 (6.6)	10.6 (6.8)	
P-value	0.018[a]	0.126	
Baseline to Day 91, dB			
Δ PTA LS means (SE)	47.8 (4.8)	41.0 (5.2)	31.1 (4.9)
Δ PTA LS mean difference (SE)	16.7 (6.8)	9.9 (7.1)	
P-value	0.016[a]	0.165	

[a]Significant at 0.04 level for AM-111 0.4 mg/ml versus placebo (post hoc), applying the weighted Bonferroni–Holm procedure. ''Intention to treat'' analysis set.

LS indicates least squares; PTA, pure-tone average (three most affected frequencies); SE, standard error. ANCOVA with baseline PTA as covariate.

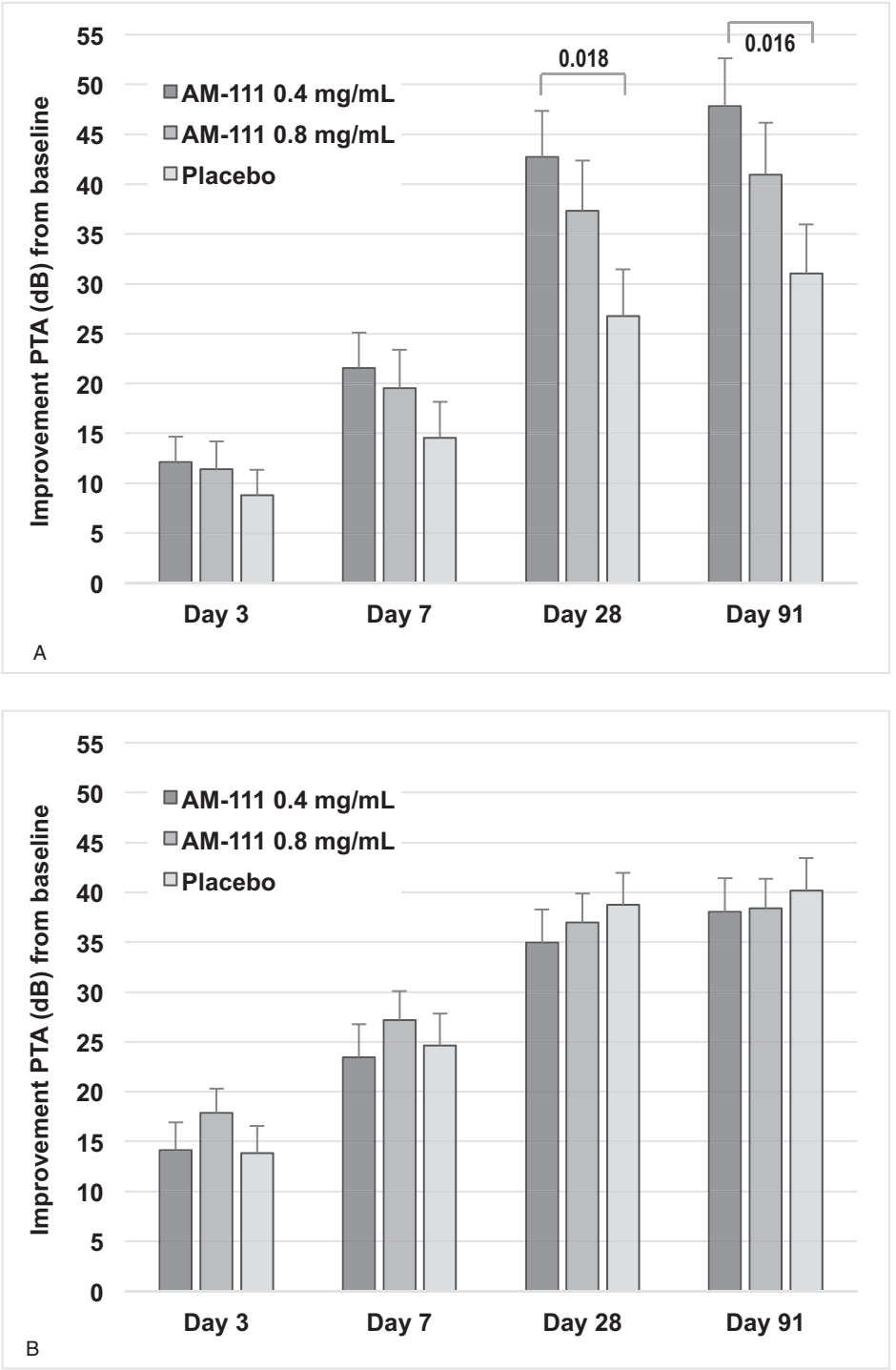

FIG. 2. Improvement of hearing threshold (least square means) at the average of the three worst affected contiguous pure-tone test frequencies from baseline (PTA) with standard error mean; post-hoc repeated measures ANCOVA, intention to treat analysis set. Significant differences between AM-111 0.4 mg/ml and placebo are shown with *p* values (post-hoc) at a significance level of 0.04, applying the weighted Bonferroni–Holm procedure. *A*, Profound acute hearing loss subpopulation (PTA $\geq$ 90 dB; n = 98). *B*, Severe acute hearing loss subpopulation (PTA 60–89 dB; n = 142).

experienced severe TEAEs of vertigo (two patients), and ear pain and blood pressure increased (one patient each). The incidence of study drug-related TEAEs was 1.2%, 4.7%, and 1.2% following AM-111 0.4 mg/ml, AM-111 0.8 mg/ml, and placebo, respectively (Table 8, column ''TR''). For procedure-related TEAEs, the incidence was 2.4%, 9.3%, and 3.5%, respectively. Nonfatal serious AEs (SAEs) were recorded for seven patients (two, two,

TABLE 5. *Use of reserve therapy in profound hearing loss subgroup*

	AM-111 0.4 mg/ml N = 35	AM-111 0.8 mg/ml N = 30	Placebo N = 33
Eligible for reserve therapy			
Yes (%)	12 (34)	11 (37)	15 (46)
No (%)	23 (66)	19 (63)	18 (54)
Received reserve therapy			
Yes (%)	16 (46)	10 (33)	13 (38)
No (%)	19 (54)	20 (67)	20 (62)
Δ PTA Day 7 to Day 28, dB			
With reserve therapy	19.9	17.6	14.2
Without reserve therapy	23.1	17.2	13.0

PTA indicates pure-tone average (three worst affected frequencies). ''Intention to treat'' analysis set.

TABLE 6. *No improvement in profound hearing loss subgroup*

	AM-111 0.4 mg/ml N = 35	AM-111 0.8 mg/ml N = 30	Placebo N = 34
Baseline to Day 28			
Δ PTA < 15 dB, n (%)	4 (11)	6 (20)	14 (41)
Relative risk ratio (95% CI)	0.278 (0.102–0.759)	0.535 (0.214–1.104)	
p value	0.006	0.105	
Baseline to Day 91			
Δ PTA < 15 dB, n (%)	4 (11)	5 (17)	13 (38)
Relative risk ratio (95% CI)	0.299 (0.108–0.826)	0.436 (0.176–1.080)	
p value	0.013	0.093	

CI indicates confidence interval; PTA, pure-tone average (three worst affected frequencies). Relative risk ratio versus placebo. ''Intention to treat'' analysis set, last observation carried forward.

and three in the AM-111 0.4 mg/ml, 0.8 mg/ml, and placebo groups, respectively). Only one SAE concerned the ears (acoustic neuroma removal; AM-111 0.8 mg/ml), and no SAE was considered to be treatment-related.

DISCUSSION

The HEALOS trial provided further confirmation that intratympanic AM-111 is well tolerated and safe. Regarding efficacy, a clinically meaningful and significant hearing recovery could be demonstrated in the profound ISSNHL subpopulation, but not in the severe ISSNHL subpopulation. This contrasts with the positive outcome observed in the preceding phase 2 trial for the combined severity categories. A priori, there were no major differences in patient demographics or hearing loss characteristics between the two trials which could explain this discrepancy. However, hearing recovery in placebo-treated HEALOS patients was higher than

TABLE 7. *Final hearing status—profound hearing loss subgroup*

	AM-111 0.4 mg/ml N = 35	AM-111 0.8 mg/ml N = 30	Placebo N = 34
PTA at Day 91, dB			
Mean (SD)	61.1 (31.7)	65.3 (27.7)	75.8 (33.5)
Median	68.0	67.0	82.0
Range	5–120	12–120	13–120
WRS at Day 91, %			
Mean WRS (SD)	56.4 (40.6)	47.7 (41.1)	45.2 (40.2)
Median	70.0	36.0	46.5
Range	0-100	0-100	0-100
Grade at Day 91			
Patients with good or serviceable hearing, %	34.3	24.1	20.6

PTA indicates pure-tone average (three worst affected frequencies); SD, standard deviation; WRS, word recognition score at 80 dB. Good or serviceable hearing according to Gardner–Robertson (Class I or Class II). ''Intention to treat'' analysis set, last observation carried forward.

expected and exceeded the level observed in the phase 2 trial, especially in the severe ISSNHL subpopulation (c. +8 dB). The spontaneous recovery in HEALOS of 36.3 dB after 3 months also exceeded the improvement of 30 to 32 dB reported for corticosteroid-treated patients with severe-profound hearing loss in other published ISSNHL studies (25,26).

HEALOS showed a severity-dependent treatment effect of AM-111 that was more accentuated than in the previous trial. As demonstrated in a murine noise trauma model, activation of the JNK pathway—AM-111's target—depends on the severity of cochlear injury (27). Whereas exposure to 110 dB or more induced activation of the JNK pathway as shown by c-Jun phosphorylation and resulted in permanent hearing loss, this was not the case with exposure to 90 dB. The presence of thresholds is a common feature of signal transduction systems such as JNK. These systems are silent as long as a certain threshold of activation is not attained and then turned ''ON'' once the required level of stimulus is reached. This has been well documented in various pathophysiological models (28–30). The lack of a treatment effect in the severe ISSNHL subpopulation suggests that there was little or no JNK activation in these patients.

In HEALOS, the improvement in hearing thresholds in the profound hearing loss subpopulation was coupled with clinically meaningful improvement in WRS. Most patients could not recognize a single word at baseline (the median was zero in all treatment groups). As expected, it took some time and hearing recovery before speech discrimination started to improve. Although of considerable clinical interest, the WRS was only an exploratory endpoint in the present study. This reflects the lack of standardization across countries and languages which differ in the spectral distribution of sound, their use of

TABLE 8. *Most commonly reported treatment-emergent adverse events by treatment group (≥ 2%)*

	AM-111 0.4 mg/ml		AM-111 0.8 mg/ml		Placebo	
	N = 85		N = 86		N = 85	
Number (%) of Patients	AC	TR	AC	TR	AC	TR
Any adverse event	28 (33)	1 (1)	23 (27)	4 (5)	29 (34)	1 (1)
Severity						
Mild	22 (26)	1 (1)	18 (21)	3 (4)	24 (28)	1 (1)
Moderate	10 (15)	0 (0)	8 (9)	1 (1)	8 (9)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	3 (4)	0 (0)
Ear and labyrinth disorders	11 (13)	1 (1)	8 (9)	1 (1)	12 (14)	1 (1)
Vertigo	5 (6)	0 (0)	6 (7)	1 (1)	5 (6)	0 (0)
Ear pain	3 (4)	0 (0)	1 (1)	0 (0)	2 (2)	0 (0)
Tinnitus	1 (1)	0 (0)	0 (0)	0 (0)	4 (5)	0 (0)
Ear discomfort	1 (1)	1 (1)	0 (0)	0 (0)	2 (2)	1 (1)
Nervous system disorders	7 (8)	0 (0)	9 (11)	1 (1)	7 (8)	0 (0)
Headache	2 (2)	0 (0)	5 (6)	0 (0)	5 (6)	0 (0)
Dizziness	3 (4)	0 (0)	4 (5)	1 (1)	1 (1)	0 (0)
Infections and infestations	7 (8)	0 (0)	6 (7)	0 (0)	6 (7)	0 (0)
Nasopharyngitis	5 (6)	0 (0)	1 (1)	0 (0)	2 (2)	0 (0)
Rhinitis	0 (0)	0 (0)	3 (4)	0 (0)	0 (0)	0 (0)
Gastrointestinal disorders	2 (2)	0 (0)	1 (1)	1 (1)	4 (5)	0 (0)
Vomiting	2 (2)	0 (0)	1 (1)	1 (1)	1 (1)	0 (0)
Nausea	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)	0 (0)
General disorders and administration site conditions	2 (2)	0 (0)	2 (2)	0 (0)	2 (2)	0 (0)
Pyrexia	0 (0)	0 (0)	1 (1)	0 (0)	2 (2)	0 (0)
Investigations	2 (2)	0 (0)	1 (1)	0 (0)	2 (2)	0 (0)
Blood pressure increased	2 (2)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)
Skin and subcutaneous tissue disorders	1 (1)	0 (0)	1 (1)	1 (1)	2 (3)	0 (0)
Rash	0 (0)	0 (0)	0 (0)	0 (0)	2 (3)	0 (0)

Adverse events by System Organ Class (Medical Dictionary for Regulatory Activities [MedDRA]).
AC indicates all causality; TR, treatment-related (study drug). ''Safety Population'' analysis set.

high-frequency sibilant sounds and of mono- or disyllabic words.

The study showed a higher share of patients recovering to good or serviceable hearing and a lower risk of no improvement in the AM-111 0.4 mg/ml group versus placebo group. This outcome seems very encouraging, especially when considering that patients with profound ISSNHL are facing a particularly poor prognosis. Studies show no improvement according to Siegel's criteria in 30% to 70% of cases, even with corticosteroid treatment (4,31,32), and such incidence being 50% to 100% higher for profound ISSNHL compared with severe ISSNHL (33).

Subjects with profound hearing loss are deaf or near-deaf on the affected ear and thus unable to understand conversational speech even when using a hearing aid. Even if only one ear is affected, speech perception, communication, and social interaction can be substantially impacted (34). Binaural hearing provides auditory cues that are critical for the processing of complex auditory signals such as speech perception in noise and localization of sound (35). An inability to determine where a sound originates can be frustrating and even disorienting to the listener, and it may also be very dangerous and put patients at risk for accidents (2).

Unlike hearing recovery, AM-111 showed no impact on tinnitus, suggesting that the two symptoms of cochlear dysfunction are generated through different pathophysiologic mechanisms. This contrasts with the observation of a higher rate of complete tinnitus remission in active-treated patients in the previous trial. In HEALOS, information on tinnitus status was collected weekly rather than only during study visits as in phase 2. This more frequent data collection coupled with the larger sample size may have resulted in more reliable and representative data; however, it may also have drawn the patient's attention more to the symptom, resulting in more awareness. Focusing away from tinnitus has been proposed as an effective approach to have the symptom recede into the background of awareness (36).

As in the previous trial, AM-111 was most effective at the concentration of 0.4 mg/ml. The smaller treatment effects for 0.8 mg/mlml in HEALOS and for 2.0 mg/ml in the previous trial suggest that the dose response curve for AM-111 is bell-shaped in humans, similar to observations in an animal noise trauma model (unpublished data). This specific shape points to more complex biological effects than observed with drugs showing ''classic'' sigmoidal dose effect relationships, which probably reflects dose-dependent inhibition of prosurvival effects

of JNK and/or induction of deleterious cell membrane perturbation. JNK inhibition protects against a plethora of neurodegenerative stimuli, yet in a different phase JNK and its nuclear substrate c-Jun also mediates essential physiological functions such as neuronal regeneration (37,38). D-TAT, AM-111's active transporter sequence, is highly positively charged to permeate the cell membrane; it has been shown that various cell-permeable peptides become cytotoxic at higher concentrations (39). Either effect may offset some part of AM-111's otoprotective effect at higher concentrations.

In conclusion, the HEALOS trial demonstrated that a single intratympanic dose of AM-111 0.4 mg/ml provides effective otoprotection in case of profound ISSNHL. Activation of the JNK stress kinase seems to require pronounced acute cochlear injury associated with large hearing threshold shifts. Further evaluation of spontaneous recovery rates in the severe ISSNHL subpopulation seems warranted as JNK activation may occur at least in parts of this subpopulation.

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