

Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology

BLA NUMBER: STN #125874.000

DATE RECEIVED BY CBER: Module 4 Received: 11-Dec-2025
Filing Letter Due (start of 60-day clock): 21-Feb-2026
BLA Action Due Date: 22-Apr-2026

DATE REVIEW COMPLETED: 18-Mar-2026

PRODUCT: OTARMENI™ (lunsotogene parvec cwha) suspension,
for intracochlear infusion

APPLICANT: Regeneron Pharmaceuticals, Inc.
PROPOSED INDICATION: OTARMENI is an adeno associated virus (AAV) vector-
based gene therapy indicated for the treatment of
pediatric and adult patients with severe-to-profound
sensorineural hearing loss with preserved outer hair cell
function and molecularly confirmed biallelic variants in
the otoferlin (*OTOF*) gene

PHARM/TOX REVIEWERS: Kate Dabirsiaghi, Lara Abramowitz
PHARM/TOX BRANCH CHIEF: Danielle Brooks
PRODUCT (CMC) REVIEWERS: Bo Liang (Chair/Lead Reviewer), Mark Verdecia,
Meghna Thakur, Bizunesh Abere, Tania Rosen-
Cheriyen, Zachary Mandell, Zhili Xu, Stella Lee, Lauren
Kokai, Johnny Lam

CLINICAL REVIEWERS: Ning Hu, Prateek Shukla
PROJECT MANAGER: Rachel Blasdel, Helen Sansone
BRANCH CHIEF: Danielle Brooks
DIVISION DIRECTOR: Allen Wensky
OFFICE DIRECTOR: Steven Fleischer

EXECUTIVE SUMMARY:

OTARMENI™ (lunsotogene parvec cwha, DB-OTO) is a dual adeno-associated virus serotype 1 (AAV1) vector-based gene therapy indicated for pediatric and adult subjects with profound sensorineural hearing loss due to biallelic mutations in the human otoferlin (*hOTOF*) gene. The therapy utilizes a split-vector approach where the *hOTOF* gene is divided into 5' and 3' components, each packaged in separate AAV1 vectors designed to co-transduce target cells and

recombine to form full-length *hOTOF* complementary DNA (cDNA). Expression is driven by a hair cell-specific promoter derived from myosin 15 (Myo15), which is designed to promote targeted protein production within the inner hair cells (IHCs) of the cochlea.

Nonclinical pharmacology studies were conducted in Otof-Q828X hom (homozygous) mice, a model of *OTOF*-related deafness that recapitulates the human phenotype with profound hearing loss and absent auditory brainstem responses (ABRs). Single intracochlear (IC) administration of DB-OTO at dose levels ranging from 1.6×10^{10} to 1.5×10^{11} vector genomes (vg)/ear resulted in dose-dependent improvements in ABR thresholds, with the highest dose level achieving mean improvements of 45-55 decibels (dB) in the mid-frequency range. At the highest dose level, the majority of mice achieved thresholds within the normal range. This functional recovery in ABR correlated with otoferlin protein expression in IHCs and was sustained through the last assessment at 30 weeks, demonstrating durable therapeutic benefit.

Biodistribution studies showed local cochlear retention of DB-OTO with minimal systemic exposure in Otof-Q828X hom mice and cynomolgus monkeys. DB-OTO vector DNA was sustained in temporal bone samples through the last study endpoint at 90 days in mice and 27 weeks in monkeys, while systemic distribution was transient with rapid clearance from plasma and cerebrospinal fluid (CSF). *hOTOF* messenger RNA (mRNA) expression was restricted primarily to cochlear tissue, with minimal, transient expression in non-otic neural tissues and no detectable expression of *hOTOF* mRNA in reproductive tissues.

Single-dose toxicology studies were conducted in Otof-Q828X hom mice and cynomolgus monkeys. The intended clinical route of administration, IC injection via the round window membrane (RWM), was used in adult mice and monkeys, while a posterior semicircular canal (PSCC) injection was used in juvenile mice to assess a worst-case scenario for systemic exposure. The 2-month Good Laboratory Practice (GLP) study in adult mice, and the 3-month GLP study in juvenile mice, at dose levels up to 1.3×10^{11} vg/ear showed no DB-OTO-related adverse findings, with a no observed adverse effect level (NOAEL) of 1.3×10^{11} vg/ear. The 6-month GLP study in monkeys at dose levels up to 4.4×10^{12} vg/ear demonstrated no DB-OTO-related toxicity, with a NOAEL of 4.4×10^{12} vg/ear. All observed adverse findings were attributed to the surgical procedure rather than the test article. In monkeys, these procedure-related findings included cases of facial paralysis, ABR threshold elevations primarily at high frequencies, and microscopic damage to inner ear structures near the injection site.

Developmental and reproductive toxicity, genotoxicity, and carcinogenicity studies were not conducted with DB-OTO. These studies were not warranted based on the IC route of administration, limited systemic exposure, target patient population, and absence of safety concerns in the toxicology studies.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of OTARMENI™. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

OTARMENI is an AAV vector-based gene therapy indicated for pediatric and adult subjects with profound sensorineural hearing loss due to mutations in the *hOTOF* gene. The therapy utilizes a dual AAV1 vector system, in which the *hOTOF* gene is split into a 5' component and a 3' component, with each segment packaged into a separate AAV1 vector. This approach is designed for both vectors to co-transduce target cells, allowing for recombination to form a full-length *hOTOF* cDNA. Expression is driven by an engineered, hair cell-specific promoter derived from *Myo15*, which is designed to promote targeted protein production within the IHCs of the cochlea.

OTARMENI is supplied as a sterile, aqueous suspension. Upon thawing, it is a clear to slightly opalescent, colorless liquid, free of visible particulates. Each single-dose vial contains an extractable volume of 0.63 mL at a nominal titer of 3.0×10^{13} vg/milliliter (mL), which is comprised of (b) (4) DB-OTO-3 and (b) (4) DB-OTO-5. The suspension is formulated with excipients including sodium phosphate, sodium chloride, sucrose, and poloxamer 188.

The recommended dose level is 7.2×10^{12} vg per ear in a total volume of 0.24 mL, administered via a single IC infusion to one or both ears during a single surgical session. OTARMENI is administered through a complex procedure conducted under general anesthesia by a surgeon experienced in IC surgery. The procedure involves performing a mastoidectomy and posterior tympanotomy to gain access to the RWM, which is then perforated. OTARMENI is delivered using specific components from the provided Administration Kit, including a Vygon Premicath® catheter attached to a BD® syringe. The catheter is inserted into the cochlea, and the suspension is infused at a controlled rate of 0.9 mL/hour (hr) for 16 minutes (mins) using a syringe pump. After the infusion is complete, the catheter remains in place for a 5-min hold period before being removed, after which the surgical openings are closed. To manage potential inflammatory and immunological responses, subjects also receive prophylactic systemic corticosteroids.

Abbreviations

AAV	Adeno-associated vector
AAV1	Adeno-associated vector serotype 1
ABR	Auditory brainstem responses
cDNA	Complementary DNA
CSF	Cerebrospinal fluid
dB	Decibel
DPOAE	Distortion product otoacoustic emissions
DRG	Dorsal root ganglia
E _{max}	Maximum effect
FVB	Friend Virus B
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
Hom	Homozygous
hOTOF	Human otoferlin
Hr	Hour

IC	Intracochlear
IHC	Inner hair cell
kHz	Kilohertz
LLOQ	Lower limit of quantification
MED	Minimum effective dose
MFA	Microfilament assembly
Min	Minute
mL	Milliliters
Myo15	Myosin 15
N/A	Not applicable
NAbs	Neutralizing antibodies
NOAEL	No observed adverse effect level
OHC	Outer hair cell
OTOF	Otoferlin
PBMC	Peripheral blood mononuclear cells
PND	Postnatal day
PSCC	Posterior semicircular canal
RWM	Round window membrane
SPL	Sound pressure level
µL	Microliter
Vg	Vector genomes
Wt	Wild type

Related File(s)

CBER IND #28864: Regeneron Pharmaceuticals, Inc.; Two Recombinant Adeno-associated Viral (AAV1) Vectors, Encoding the 5' Component and 3' Component of the Human Otoferlin Protein (OTOF) Isoform 5 delivered via intracochlear injection using Vygon Premicath 1FR/28G [DB-OTO]; For Treatment of Congenital Auditory Neuropathy Secondary to Biallelic Mutations of the Otoferlin Gene. **ACTIVE**.

Table of Contents

INTRODUCTION	5
NONCLINICAL STUDIES.....	5
PHARMACOLOGY STUDIES.....	8
Summary List of Pharmacology Studies	8
SAFETY PHARMACOLOGY STUDIES	13
PHARMACOKINETIC STUDIES.....	13
Summary List of Pharmacokinetics Studies	13
Overview of Pharmacokinetic Studies	14

TOXICOLOGY STUDIES	15
Summary List of Toxicology Studies	15
Nonclinical Rationale for Clinical Dose Level Selection:	27
APPLICANT’S PROPOSED LABEL	27
CONCLUSION OF NONCLINICAL STUDIES	27
KEY WORDS/TERMS	27
REFERENCES	27

INTRODUCTION

OTOF-related deafness is an ultra-rare form of congenital, permanent hearing loss caused by biallelic pathogenic variants in the *OTOF* gene. Globally, the prevalence of congenital deafness is 1 to 2 per 1,000 children, with single-gene variants accounting for over half of these cases (Morton, 2006). In the USA, it is estimated that approximately 50 of the 6,100 new cases of congenital deafness each year are due to *OTOF* variants (Martin, 2024). The deafness results from dysfunction of the synapse between sensory IHCs and the vestibulocochlear nerve, as the otoferlin protein is critical for synaptic transmission (Pangrsic, 2012).

Patients with *OTOF*-related deafness are typically born with severe-to-profound hearing loss and are unable to hear even very loud sounds (Ford, 2023). A natural history analysis developed by the applicant, which included a meta-analysis and a retrospective chart review of over 300 subjects, indicated that individuals with *OTOF*-related deafness do not experience spontaneous recovery of their hearing. This level of congenital deafness is associated with significant impairments in language development (Yoshinaga-Itano, 1998), cognitive development (Kronenberger, 2013), academic achievement (Marschark, 2015), and reading skills (Antia, 2020).

There are no approved drug products for *OTOF*-related deafness. Current management relies on assistive devices such as hearing aids or cochlear implants, which provide sound amplification or electrical stimulation, respectively, rather than restoring the natural hearing pathway. DB-OTO is a gene therapy that is designed to address the underlying genetic cause of the condition by delivering a functional copy of the *OTOF* gene to the IHCs. It is intended to restore the expression of the otoferlin protein, thereby re-establishing synaptic function and enabling natural acoustic hearing.

NONCLINICAL STUDIES

Nonclinical Lots of DB-OTO:

The DB-OTO lots used in the non-GLP studies, DB-OTO-PHAR-017 and DB-OTO-PHAR-023, as well as the three GLP studies were manufactured using the same plasmids and manufacturing process used to produce the clinical material. All nonclinical studies used the clinical formulation

buffer, maintaining consistency with the intended clinical product. The nonclinical studies used non-GMP (Good Manufacturing Practice) material, while the clinical product is manufactured under GMP conditions. However, the nonclinical material was determined to be comparable to the clinical investigational drug product based on the process consistency. The same non-GMP lot (B564TOXP1) was used across all three GLP studies to ensure consistency in the pivotal safety and activity studies.

Comparison of Delivery Device and Administration Procedure:

The surgical delivery procedures across species demonstrate progressive refinement toward the clinical approach, with postnatal day (PND) 14-16 mice receiving DB-OTO via PSSC injection, while ≥ 5 -week-old mice, cynomolgus monkeys, and humans all receive delivery through the RWM via transmastoid/facial recess approach (Table 1). Key procedural differences include the use of fenestration (absent in mice but present in monkeys and humans), bilateral dosing in monkeys and humans versus unilateral in mice, and the inclusion of perioperative antibiotics and postoperative corticosteroids in monkeys and humans but not in mice. The delivery volumes are scaled appropriately across species (2 μL [microliters] in mice, 60 μL in monkeys, 240 μL in humans) with injection rates ranging from manual delivery in mice to controlled pump delivery at 15 $\mu\text{L}/\text{min}$ in monkeys and humans.

Table 1: Species Comparison of Surgical Delivery Procedures

	PND 14-16 Mice	≥ 5-Week-Old Mice	Cynomolgus Monkeys	Humans
Surgical Approach	Postauricular	Transbullae	Transmastoid/facial recess	Transmastoid/facial recess
Catheter Placement	Posterior semicircular canal	RWM	RWM	RWM
Fenestration	None	None	Yes, lateral semicircular	Yes, lateral semicircular
Unilateral/Bilateral	Unilateral	Unilateral	Bilateral	Unilateral or bilateral
Catheter	Polyimide microfilament	Polyimide microfilament	Vygon Premicath 1Fr/28G, 30 cm (1261.30)	Vygon Premicath 1Fr/28G, 30 cm (1261.30)
Syringe	Glass microcapillary directly connected to injector pump head	1 mL Luer-Lok Syringe	BD 1 mL Luer-Lok Syringe (309628)	BD 1 mL Luer-Lok Syringe (309628)
Pump	(b) (4) injector (b) (4)	N/A (manually)	(b) (4) syringe pump	(b) (4) syringe pump
Rate	0.2 µL/min	Manually (approximately 4 µL/min)	15 µL/min (0.9 mL/hr)	15 µL/min (0.9 mL/hr)
Volume	2 µL (1.2 × average perilymph volume)	2 µL (1.2 × average perilymph volume)	60 µL (1.5 × average perilymph volume)	240 µL (1.5 × average perilymph volume)
Duration of Injection	10 min	Approximately 30 s (manually controlled)	4 min	16 min
Contrast Agent	0.04% trypan blue	0.04% trypan blue	No	No
Perioperative Antibiotics	No	No	Yes	Yes
Postoperative Corticosteroids	No	No	Yes	Yes

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to demonstrate biological activity and to support the rationale for the IC administration of DB-OTO in the proposed clinical indication.

In Vivo Studies in Otof-Deficient Mouse Models

Study Number	Study Title	Report Number
1	Characterization of the Hearing Phenotype and Hair Cell Otoferlin Expression in the Otof-Q828X Mouse Model	DB-OTO-PHAR-003
2	3-Month Dose Range Finding and Efficacy Study of DB-OTO in Otoferlin Deficient Mice	DB-OTO-PHAR-017
3	8-Month Dose Range Finding and Efficacy Study of DB-OTO in <i>Otof</i> -Deficient Mice	DB-OTO-PHAR-023

Note: Study No. 1 is not reviewed in this memo because it is a characterization study of the Otof-Q828X hom (homozygous) mouse model. However, the description of the Otof-Q828X hom mouse model in the '*In Vivo Studies in Otof-Deficient Mouse Models*' section is based on data from Study No. 1.

In Vivo Studies in Otof-Deficient Mouse Models

Otof-Q828X Hom Mouse Model:

The Otof-Q828X hom mouse model was generated using CRISPR-Cas9-mediated knock-in to introduce a Q828X nonsense mutation in the mouse *Otof* gene, which is orthologous to the Q829X mutation in the human *OTOF* gene that causes congenital deafness. The mice show profound hearing loss from the expected time of onset of normal hearing development that occurs around 2 weeks postnatally, which mimics human congenital deafness due to *OTOF* deficiency.

ABR thresholds are minimal or absent at all ages tested, which is consistent with the complete loss of detectable otoferlin protein expression in IHCs. This ABR phenotype mirrors that observed in human subjects with biallelic *OTOF* mutations, who typically present with absent or highly abnormal ABRs due to defective IHC synapses with the auditory nerve. In contrast to their severe hearing impairment, Otof-Q828X hom mice initially maintain normal distortion product otoacoustic emission (DPOAE) thresholds across frequencies through 24 weeks of age, indicating that outer hair cells (OHCs) remain functional early in life. After 24 weeks, these mice show accelerated loss of DPOAE responses compared to wild type (wt) and heterozygous mice, suggesting progressive deterioration of OHC function over time. Similarly, human subjects with *OTOF* variants generally have present DPOAE responses when initially diagnosed as infants, indicating functional OHCs despite the IHC dysfunction. However, these subjects lose DPOAE responses over time during childhood, with variable rates of progression.

Histologically, the mice retain the majority of both IHCs and OHCs throughout the studied time course of 43 weeks, although there is moderate, progressive loss of both hair cell types with age.

Mean IHC counts are reduced by a maximum of 30% at the oldest ages. The model demonstrates complete absence of otoferlin protein expression in IHCs while maintaining sufficient numbers of target hair cells, making it an appropriate model to evaluate the ability of DB-OTO to restore otoferlin protein expression and hearing function in the target cell population.

Study #2

Report Number		DB-OTO-PHAR-017
Date Report Signed		01-Sep-2022
Title		3-Month Dose Range Finding and Efficacy Study of DB-OTO in Otoferlin Deficient Mice
GLP Status		No
Testing Facility		Decibel Therapeutics 1325 Boylston Street, Suite 500 Boston, MA 02215
Objective(s)		To evaluate the pharmacology of DB-OTO 12 weeks post-administration at doses ranging from 1.6×10^{10} to 1.5×10^{11} vg/ear in Otof-Q828X hom and Otof-wt mice
Study Animals	Strain/Breed	Otof-wt Friend Virus B (FVB) Otof-Q828X hom (Background strain: FVB) Note: Both mice are FVB mice. Otof-wt is the wild type FVB mouse, while Otof-Q828X hom is the gene edited <i>Otof</i> knockout FVB mouse
	Species	Mouse (<i>Mus musculus</i>)
	Age	5-14 weeks old
	Body Weight	17.2-34.1 g
	#/sex/group	3-10 animals/sex/group
	Total #	112 animals
Test Article(s)		DB-OTO (Lot # 564-0321-342 and 564-0421-371)
Control Article(s)		Diluent (0.001% poloxamer 188 LOW) Diluent ((b) (4) poloxamer 188 HIGH) Note: Post-production testing revealed that the poloxamer 188 concentration was ((b) (4)) instead of the expected 0.001%; the clinical formulation includes 0.001% poloxamer 188. The applicant addressed this by including control groups with both poloxamer concentrations (0.001% and ((b) (4))) to demonstrate that the increased concentration did not have significant effects on hearing measures. The applicant did not explain how this manufacturing error occurred.
Route of Administration		IC injection via the RWM
Description of the Surgical/Administration Procedure		DB-OTO was administered to mice via a single, unilateral IC injection into the left ear via the RWM. The applicant does not provide additional details in the study report. However, some additional information was provided in Table 1.

Study Groups and Dose Levels	<p>Group 1: 1.5×10^{11} vg/ear DB-OTO (Otof-Q828X hom)</p> <p>Group 2: 7.2×10^{10} vg/ear DB-OTO (Otof-Q828X hom)</p> <p>Group 3: 1.6×10^{10} vg/ear DB-OTO (Otof-Q828X hom)</p> <p>Group 4: 1.5×10^{11} vg/ear DB-OTO (Otof-wt)</p> <p>Group 5: 1.1×10^{11} vg/ear DB-OTO (Otof-wt)</p> <p>Group 6: 7.2×10^{10} vg/ear DB-OTO (Otof-wt)</p> <p>Group 7: 3.6×10^{10} vg/ear DB-OTO (Otof-wt)</p> <p>Group 8: 1.6×10^{10} vg/ear DB-OTO (Otof-wt)</p> <p>Group 9: No injection (Otof-wt)</p> <p>Group 10: Diluent (0.001% poloxamer 188 LOW) (Otof-wt)</p> <p>Group 11: Diluent (b) (4) poloxamer 188 HIGH) (Otof-wt)</p> <p>DB-OTO was administered in (b) (4) poloxamer 188 HIGH in Groups 1-8. Injection volume was 2 μL for all groups.</p>
Dosing Regimen	Single unilateral administration
Randomization	No
Description of Masking	The applicant describes blinded assessments of ABR thresholds as well as blinded manual counting of IHCs and OHCs.
Scheduled Sacrifice Time Points	<p>Week 12-16</p> <p>Note: Individual animals were sacrificed between 12 and 16 weeks after injection. While the intended sacrifice time point was 12 weeks, this range reflects the logistical constraints of terminal evaluations of 112 animals across 11 groups in this non-GLP study.</p>

Key Evaluations and Assessments:

- ABR and DPOAE: Weeks 2 (Otof-wt mice only), 4, 8, and 12
- Vector biodistribution and *hOTOF* mRNA expression (via in situ hybridization): Week 12-16
- Human otoferlin protein expression in IHCs (via immunohistochemistry): Week 12-16
- IHC and OHC counts: Week 12-16
- Histology: Week 12-16

Key Results:

- A vehicle control arm of the study demonstrated that a higher-than-expected concentration of poloxamer 188 (b) (4) vs. 0.001%) in the formulation buffer had no significant effect on ABR or DPOAE thresholds in Otof-wt mice.
- A total of 10/112 mice died during the study. These deaths were not attributed to DB-OTO, as mortality was similarly distributed across all DB-OTO, vehicle, and naive groups and was considered related to repeated anesthesia.
- Administration of DB-OTO resulted in significant, dose-dependent improvements in ABR thresholds in Otof-Q828X hom mice. The highest dose level of 1.5×10^{11} vg/ear led to the largest hearing improvements of 45 to 55 dB in the mid-frequency range, while the lowest dose level of 1.6×10^{10} vg/ear resulted in improvements of approximately 25 dB. The majority of Otof-Q828X hom mice administered DB-OTO at 1.5×10^{11} vg/ear achieved ABR thresholds within the normal range of control Otof-wt mice when

evaluated at the 22.6 kilohertz (kHz) frequency (58% in normal range at Week 4, 68% at Week 8, and 63% at Week 12).

- The minimum effective dose (MED) in this study was determined to be 7.2×10^{10} vg/ear, which is based on the applicant's MED criteria requiring ABR thresholds < 80 dB at two or more frequencies in over 75% of animals. At this dose level, 89% of mice (16/18) were considered responders, whereas the lower 1.6×10^{10} vg/ear dose level did not meet this threshold, with a 56% response rate (5/9 mice).
- The observed hearing recovery was dose-dependent and correlated with the expression of the otoferlin protein. The highest dose group showed the greatest number of otoferlin-positive IHCs.
- The integrity of the cochlea, as measured by DPOAE, was generally normal in most animals (85/102 surviving animals). The severe DPOAE loss observed in a minority of animals (17/102) was attributed to the surgical procedure, as it was not dose-dependent and was distributed across all groups, including controls.
- Histopathological evaluation at Week 12-16 showed no DB-OTO-related findings in the middle and inner ears or in systemic tissues, including the heart, liver, spleen, kidney, and brain.
- In Otof-wt mice with normal hearing, administration of DB-OTO at dose levels up to 1.5×10^{11} vg/ear did not cause any significant changes in ABR thresholds compared to vehicle-administered animals, indicating that DB-OTO was well-tolerated in the presence of the endogenous otoferlin protein.

Reviewer Comments:

- *The study demonstrated a dose-dependent restoration of auditory function in the Otof-Q828X hom mouse model, with ABR threshold improvements of 45-55 dB compared to controls at the highest dose level. This functional recovery correlated with otoferlin protein expression in IHCs, supporting the intended mechanism of action. The data provides nonclinical mechanistic evidence for DB-OTO by demonstrating biological activity and the potential for clinically meaningful hearing improvement in subjects with OTOF-related hearing loss.*
- *A notable limitation is the incidence of severe cochlear damage (DPOAE loss) in a subset of animals (17 of 102 surviving mice), which was attributed to the IC surgical procedure. This procedural variability introduces a confounding factor in assessing the full activity of DB-OTO and highlights a potential risk associated with the delivery method.*

Study #3

Report Number	DB-OTO-PHAR-023
Date Report Signed	23-Aug-2022
Title	8-Month Dose Range Finding and Efficacy Study of DB-OTO in Otof-Deficient Mice
GLP Status	No
Testing Facility	Decibel Therapeutics 1325 Boylston Street, Suite 500 Boston, MA 02215

Objective(s)		To evaluate the pharmacology of DB-OTO for up to 30 weeks following a single IC injection into male and female Otof-Q828X hom mice
Study Animals	Strain/Breed	Otof-Q828X hom (Background strain: FVB)
	Species	Mouse (<i>Mus musculus</i>)
	Age	6.1–12.6 weeks
	Body Weight	18.6–29.2 g
	#/sex/group	4-6 animals/sex/group
Total #		30 animals total
Test Article(s)		DB-OTO (Lot # 564-0221-296 and 564-0321-324)
Control Article(s)		Diluent (0.001% poloxamer 188)
Route of Administration		IC injection via the RWM
Description of the Surgical/Administration Procedure		The animals were anesthetized and a postauricular incision was made, followed by exposure of the tympanic bulla and creation of a small hole to visualize the RWM. The RWM was then punctured using a machine-pulled glass capillary or microfilament assembly (MFA). The MFA was attached to a preloaded syringe and was inserted into the RWM at a depth of approximately 1 mm. A 2 µL volume of DB-OTO mixed with trypan blue contrasting agent was manually delivered over approximately 30 seconds into the scala tympani space of the cochlea. Lastly, the hole in the bulla was sealed with muscle tissue and the skin incision was closed with topical tissue glue.
Study Groups and Dose Levels		Group 1: 5.4×10^{10} vg/ear DB-OTO Group 2: 3.6×10^{10} vg/ear DB-OTO Group 3: 1.8×10^{10} vg/ear DB-OTO Note: There was no control group included. The applicant indicated that ABR and DPOAE thresholds were compared to values from 8-to-44-week-old uninjected Otof-Q828X hom mice (59 ABR and 73 DPOAE measurements) from an early characterization study (Study DB-OTO-PHAR-003).
Dosing Regimen		Single unilateral administration
Randomization		No
Description of Masking		The applicant describes blinded assessments of ABR thresholds as well as blinded manual counting of IHCs and OHCs.
Scheduled Sacrifice Time Points		Week 30

Key Evaluations and Assessments:

- ABR and DPOAE: Weeks 2, 4, 8, 12, and 30
- Human otoferlin protein expression in IHCs (via immunohistochemistry): Week 30
- IHC and OHC counts: Week 30

Key Results:

- A total of 4/30 mice died during the study. These deaths were not attributed to DB-OTO, as this mortality rate was similar to that observed in other DB-OTO mouse studies. All deaths were in DB-OTO groups, as there were no control groups in this study.
- Progressive loss of DPOAE responses was observed over 30 weeks in DB-OTO-administered animals, similar to control animals in prior studies. Within-animal comparison of DPOAE responses in the DB-OTO-administered ears versus uninjected contralateral ears suggests DB-OTO might modestly slow this progression.

- DB-OTO resulted in sustained otoferlin protein expression in IHCs and durable recovery of ABR thresholds up to 30 weeks post-administration. ABR thresholds in DB-OTO-administered Otof-Q828X hom mice generally improved across all frequencies, with the largest improvement observed at 22.6 kHz. Approximately half of DB-OTO-administered animals at the two highest dose levels (3.6×10^{10} and 5.4×10^{10} vg/ear) achieved ABR thresholds within the range of naive Otof-wt animals at 22.6 kHz, though direct dose comparisons were limited by procedure-related hearing loss in a subset of animals, particularly in the highest dose group.
- Histological assessments of Otof-Q828X hom animals showed that OHC and IHC counts were consistent with counts from age-matched naive Otof-Q828X hom mice and were unaffected by DB-OTO administration or dose level.

Reviewer Comments:

- *This study demonstrated that a single IC administration of DB-OTO resulted in sustained otoferlin protein expression in IHCs and durable ABR threshold improvements that were maintained for at least 30 weeks post-administration. This provides the longest durability data in the DB-OTO nonclinical development program and supports the potential for long-term therapeutic benefit in subjects with OTOF-related hearing loss.*
- *The within-animal comparison of DB-OTO-administered versus uninjected contralateral control ears at 30 weeks suggests that DB-OTO administration may modestly slow the progression of DPOAE loss. DB-OTO-administered ears showed median thresholds that were approximately 11 dB improved compared to their contralateral control ears, which supports the potential for DB-OTO to slow hearing loss progression in OTOF-deficient subjects.*

SAFETY PHARMACOLOGY STUDIES

No standalone safety pharmacology studies were conducted with DB-OTO. However, the toxicology studies included some safety pharmacology endpoints and are reviewed in the 'Toxicology Studies' section.

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetics Studies

The following analytical methods and validation reports were generated to support the analysis performed for the pharmacokinetic evaluations, and the following pharmacokinetic studies were conducted.

Analytical Methods and Validation Reports

Study Number	Study Title	Report Number
4	Validation of a Polymerase Chain Reaction Method for the Quantitative Determination of Vector DNA in Cynomolgus Tissues, Plasma, CSF, and Shedding Samples	20323816
5	Validation of a Polymerase Chain Reaction Method for the Quantitative Determination of Vector DNA in Mouse Tissues and Plasma	20323822
6	Quantitative Reverse Transcriptase Polymerase Chain Reaction Assay for the Quantitative Determination of <i>hOTOF</i> RNA Expression in Cynomolgus Monkey Tissues Applying Absolute Quantification	AP-20323817-MB-04
7	Quantitative Reverse Transcriptase Polymerase Chain Reaction Assay for the Quantitative Determination of <i>hOTOF</i> RNA Expression in Mouse Tissues Applying Absolute Quantification	AP-20323823-MB-01
8	Validation of an (b) (4) Method for the Detection of Antibodies against Otoferlin in Cynomolgus Serum	BAL-20-715-023
9	Qualification of an (b) (4) Method for the Detection of Antibodies against Otoferlin in Cynomolgus Cerebrospinal Fluid	BAL-20-715-024
10	Validation of a Cell Based Assay to Detect Neutralizing Antibodies Against AAV1 in Cynomolgus Serum	BAL-20-715-027
11	Qualification of a Cell Based Assay to Detect Neutralizing Antibodies Against AAV1 in Cynomolgus Cerebrospinal Fluid	BAL-20-715-028
12	Qualification of an IFN- γ (b) (4) Assay to Detect Antigen-Specific Immune Activation to DB-OTO in Mouse Splenocytes	BAL-20-715-036
13	Qualification of an IFN- γ (b) (4) Assay to Detect Antigen-Specific Immune Activation to DB-OTO in Cynomolgus (Non-Human Primate) PBMCs	BAL-20-715-038

Note: Study Nos. 4-13 are not reviewed in this memo because they are analytical methods and validation reports that support the various methods and assays used throughout the nonclinical studies evaluating DB-OTO.

In Vivo Studies

Study Number	Study Title	Report Number
14	Timing of Human <i>OTOF</i> Transgene Expression in DB-OTO Transduced Mice	DB-OTO-PHAR-012
15	Kinetics of Expression of the Human Otoferlin Transgene in the Inner Ears of Cynomolgus Monkeys Transduced with DB-OTO	DB-OTO-PHAR-010

Note: Study Nos. 14-15 are summarized in the ‘*Overview of Pharmacokinetic Studies*’ section but are not reviewed in this memo because they were conducted to inform the design of GLP pharmacology, biodistribution, and toxicity studies which are reviewed in detail in the ‘*Toxicology Studies*’ section.

Overview of Pharmacokinetic Studies

Following administration of DB-OTO to Otof-Q828X hom mice and cynomolgus monkeys, vector genomes were detected in all injected ears with dose-dependent increases and persistence through study endpoints. Systemic distribution was limited with transient detection in plasma

and CSF that cleared by 7 weeks in monkeys. Expression of *hOTOF* mRNA was detectable within 3 days in mice and 2 weeks in monkeys, reaching peak levels at 4-6 weeks and plateauing through 8 weeks in both species. *hOTOF* mRNA expression was primarily restricted to cochlear tissue in both species, with minimal and transient expression in non-otic neural tissues at levels 5-96-fold lower than cochlear expression. There was no detectable expression of *hOTOF* mRNA in reproductive tissues. Otoferlin protein expression was observed in IHCs of DB-OTO administered mice, demonstrating functional transgene translation. Viral shedding was detected in multiple samples (buccal swabs, nasal swabs, feces, and urine) on Day 2 post-administration in monkeys but was absent by 7 weeks, indicating clearance from excretory pathways.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of DB-OTO following IC administration in Otof-Q828X hom mice and cynomolgus monkeys.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
16	A 2-Month GLP Pharmacology, Toxicity, and Biodistribution Study in Adult Otof-Q828X Homozygote Mice	OTOF-10104
17	A 3-Month GLP Toxicity and Biodistribution Study in Homozygous Otof-Q828X Mice by Inner Ear Injection with DB-OTO	OTOF-10088
18	A 7- and 27-Week Toxicity and Biodistribution Study Following a Single Intracochlear Injection of DB-OTO in Cynomolgus Monkeys	OTOF-10091

Developmental and Reproductive Toxicology Studies:

No reproductive or developmental toxicity studies have been conducted with DB-OTO. To support risk assessments for developmental and reproductive toxicity, the biodistribution of DB-OTO vector DNA and expression of *hOTOF* mRNA in the ovary and testes was assessed in a 6-month GLP study in cynomolgus monkeys (OTOF-10091) and in two studies in juvenile and adult Otof-Q828X hom mice (OTOF-10088 and OTOF-10104, respectively). Across these nonclinical studies, there was negligible biodistribution of DB-OTO vector to the gonads, no expression of *hOTOF* mRNA in the gonads, and no gross or microscopic findings in the gonads, suggesting a low reproductive and germline integration risk.

Genotoxicity Studies:

No genotoxicity studies have been conducted with DB-OTO. The risk of insertional mutagenesis with recombinant AAV vectors is considered to be low. Biodistribution studies have shown limited DB-OTO vector distribution beyond the local compartment of the inner ear, and no signs of abnormal cellular growth or tissue hyperplasia have been identified in any of the studies where juvenile or adult Otof-Q828X homologous mice or cynomolgus monkeys were administered DB-OTO.

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity studies have been conducted with DB-OTO. These were considered not warranted based on a review of the animal toxicology studies, data from biodistribution studies showing limited DB-OTO vector distribution beyond the local compartment of the inner ear, and a lack of clinical evidence of carcinogenesis attributable to recombinant AAV vectors.

Toxicology Studies**Study #16**

Report Number		OTOF-10104
Date Report Signed		21-Mar-2023
Title		A 2-Month GLP Pharmacology, Toxicity, and Biodistribution Study in Adult Otof-Q828X Homozygote Mice
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To evaluate the pharmacology, toxicity, and biodistribution of DB-OTO following peak transgene expression of <i>hOTOF</i> mRNA when delivered to the cochlea of a mature ear
Study Animals	Strain/Breed	Otof-Q828X hom (Background strain: FVB)
	Species	Mouse (<i>Mus musculus</i>)
	Age	~6-7 weeks old
	Body Weight	16.10-28.76 g
	#/sex/group	5-6 animals/group/sex
	Total #	147 animals total
Test Article(s)		DB-OTO (Lot No. B564TOXP1)
Control Article(s)		Diluent (0.001% poloxamer 188)
Route of Administration		IC injection via the RWM
Description of the Surgical/Administration Procedure		Under isoflurane anesthesia, a post-auricular incision was made to access the auditory bulla, where a small hole was drilled to expose the round window niche. The RWM was first perforated, and after perilymph leakage ceased, a custom MFA assembly was used to manually inject 2 µL of the test article or vehicle directly into the round window niche. Dose completion was visually confirmed using a colored delivery agent, after which the bulla was sealed with muscle tissue, the surgical incision was closed, and animals were monitored on a warming pad until fully recovered.
Study Groups and Dose Levels		<p>Pharmacology and Toxicology Arm: <u>Group 1:</u> Vehicle control (2 µL) <u>Group 2:</u> 1.3×10^{11} vg/ear (2 µL) <u>Group 3:</u> 8.0×10^{10} vg/ear (2 µL) <u>Group 4:</u> 4.0×10^{10} vg/ear (2 µL)</p> <p>Biodistribution Arm: <u>Group 5:</u> Vehicle control (2 µL) <u>Group 6:</u> 1.3×10^{11} vg/ear (2 µL) <u>Group 7:</u> 8.0×10^{10} vg/ear (2 µL) <u>Group 8:</u> 4.0×10^{10} vg/ear (2 µL)</p>
Dosing Regimen		Single unilateral administration
Randomization		Yes

Description of Masking	Personnel performing test and control article administration and electrophysiological measurements (ABR, DPOAE) were blinded to the identity of these articles during their administration and during electrophysiological measurement procedures.
Scheduled Sacrifice Time Points	Days 30 and 60

Key Evaluations and Assessments:

- Moribundity/mortality health checks: Twice daily
- Incision site observations: Daily until healed
- Clinical observations: Daily for 14 days, then weekly
- Body weights: Weekly
- Modified Irwin test/functional observational battery: Days 30 and 60
- Ophthalmic examinations: Days 30 and 60
- Electrophysiological measurements (ABR and DPOAE): Days 30 and 60
- Clinical pathology (hematology and clinical chemistry): Days 30 and 60
- DNA biodistribution and *hOTOF* mRNA expression: Days 30 and 60
- Gross necropsy, organ weights, and histology: Days 30 and 60

Key Results:

- There were no DB-OTO related effects on general health, body weight, ophthalmic exams, neurological assessments, or clinical pathology parameters. No DB-OTO-related gross pathological or histopathological findings were observed in non-otic tissues at either sacrifice time point.
- At the highest dose level (1.3×10^{11} vg/ear), DB-OTO-administered animals showed statistically significant improvements in ABR thresholds across most tested frequencies on Days 30 and 60 when compared to vehicle controls. The lower and mid-dose levels also demonstrated ABR threshold improvements, though these effects were less consistent across all frequencies and time points. The largest improvements occurred in the mid-frequency range (16 to 22.6 kHz), where mean ABR thresholds in all DB-OTO dose groups improved by over 50 dB in at least one sex and time point cohort, with some cohorts showing mean improvements of up to 70 dB relative to the vehicle control group.
- Click ABR thresholds, which activate hair cells along the length of the cochlea, showed improvements that were comparable to the tone ABR thresholds across all dose groups.
- DPOAE thresholds were generally consistent between DB-OTO-administered animals and controls, suggesting there were no DB-OTO-related effects on cochlear integrity.
- Severe DPOAE loss (thresholds ≥ 55 dB SPL [sound pressure level] between 8 and 16 kHz) was observed in 22% of animals. As this finding was randomly distributed across all dose level groups, including vehicle controls, it was attributed to the surgical delivery procedure.
- Measurable levels of DB-OTO vector DNA and *hOTOF* mRNA were found in all cochleae of DB-OTO-administered animals on Days 30 and 60 in a dose-dependent manner.
- In Group 6 animals, one or more animals had measurable levels of vector DNA in adrenal gland, brain (both sides), eye, kidney, liver, lungs, parotid lymph node, spleen, and testis

tissues at Day 30; and in adrenal gland, brain (both sides), cervical dorsal root ganglia (DRG), eye, kidney, liver, parotid lymph node, cervical spinal cord, and spleen at Day 60. Heart and ovary samples had no measurable levels of vector DNA at either time point. Lung and testis samples had no measurable levels of vector DNA by Day 60.

- In Group 6 animals, measurable levels of *hOTOF* mRNA were found in a subset of brain (5/9 injected side and 4/9 contralateral side), cervical DRG (5/10), and cervical spinal cord (1/10) on Day 30; and brain (4/10 injected side and 4/10 contralateral side) and cervical DRG (3/6) on Day 60. All samples isolated from non-neural tissue types (adrenal gland, eye, kidney, liver, lungs, parotid lymph node, spleen and testis) had no measurable levels of *hOTOF* mRNA expression on Days 30 or 60.
- On Day 30, minimal to mild mononuclear cell infiltrates within the inner ear were observed in 7/12 Group 4, 3/12 Group 3, and 4/12 Group 2 animals. On Day 60, this finding persisted in Group 2 and Group 3 animals but resolved in Group 4 animals. Mild vacuolization of the saccular macula was observed in a single Group 2 animal on Day 30; this finding was not observed on Day 60.
- Procedure-related microscopic findings were observed on Day 30 and 60 in both control and DB-OTO administered animals. These findings consisted of minimal to mild fibrosis and thickening of the RWM with macrophage infiltrates that were centered on bone fragments and foreign material, minimal inflammatory cell infiltrates that were centered on foreign material or bony fragments in the mucoperiosteum of the middle ear soft tissue that surrounded the middle ear bony margins, and minimal to mild macrophage infiltrates in the middle ear cavity in proximity to the surgical site.
- The NOAEL in this study for DB-OTO was determined to be the highest dose level evaluated, 1.3×10^{11} vg/ear.

Reviewer Comments:

- *The study provides mechanistic evidence for DB-OTO by demonstrating that a single IC injection results in a dose-dependent and durable restoration of auditory function compared to vehicle controls, with ABR threshold improvements of up to 70 dB. However, the hearing recovery was most pronounced in the mid-frequency range, suggesting that the degree of clinical benefit in humans may also vary across the hearing spectrum.*
- *The study establishes a favorable systemic safety profile for DB-OTO, with the NOAEL identified as the maximum feasible dose (1.3×10^{11} vg/ear). The limited biodistribution and lack of off-target tissue *hOTOF* mRNA expression, particularly in gonadal tissues, provide support for the safety of the proposed clinical dose level and mitigate concerns regarding systemic toxicity and germline transmission.*
- *Procedure-related complications, including severe hearing loss (as measured by DPOAE) in 22% of all animals, were observed across all groups, including controls. The applicant used a surgical approach through the RWM, which was similar to the clinical approach, thus providing relevant data that the surgical delivery procedure itself carries inherent risks independent of the drug product.*

Study #17

Report Number		OTOF-10088
Date Report Signed		17-Mar-2023
Title		A 3-Month GLP Toxicity and Biodistribution Study in Homozygous Otof-Q828X Mice by Inner Ear Injection with DB-OTO
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To evaluate safety and biodistribution of DB-OTO following peak and persisting transgene expression of <i>hOTOF</i> mRNA when delivered to the cochlea in P14-P16 male and female Otof-Q828X hom mice
Study Animals	Strain/Breed	Otof-Q828X hom (Background strain: FVB)
	Species	Mouse (<i>Mus musculus</i>)
	Age	~2 weeks old (P14-P16)
	Body Weight	5.74 – 10.20 g
	#/sex/group	Toxicology: 8-12 animals/sex/group/time point Biodistribution: 3-6 animals/sex/group/time point
	Total #	185 animals total
Test Article(s)		DB-OTO (Lot No. B564TOXP1)
Control Article(s)		Diluent (0.001% poloxamer 188)
Route of Administration		Inner ear via PSSC administration Note: The applicant reports the PSSC route was intentionally chosen for this study because it results in greater vector distribution outside the ear, thus enabling a more robust assessment of general toxicity in developing non-otic tissues and representing a worst-case scenario for systemic exposure.
Description of the Surgical/Administration Procedure		Under isoflurane anesthesia, a small vertical incision was made behind the left external ear, and the tissue was gently separated to access the PSSC. A burr hole was carefully drilled in the PSSC bone with bleeding controlled using sterile absorbent cotton. An MFA assembly connected to a nanoliter injector pump was inserted into the hole and sealed with tissue glue, and 2 µL of test article or vehicle was injected at a rate of 200 nanoliters/min. Following injection, the polyimide tubing was pinched, bent, and cut close to the canal to prevent leakage, tissue glue was applied to the cut end if needed, and the wound was closed using PDS sutures.
Study Groups and Dose Levels		Toxicology Arm: <u>Group 1:</u> Vehicle control (2 µL) <u>Group 2:</u> 1.3×10^{11} vg/ear (2 µL) <u>Group 3:</u> 9.2×10^{10} vg/ear (2 µL) Biodistribution Arm: <u>Group 4:</u> Vehicle control (2 µL) <u>Group 5:</u> 1.3×10^{11} vg/ear (2 µL) <u>Group 6:</u> 9.2×10^{10} vg/ear (2 µL)
Dosing Regimen		Single unilateral administration
Randomization		Yes
Description of Masking		Personnel performing test and control article administration and electrophysiological measurements (ABR, DPOAE) were blinded to the identity of these articles during their administration and during electrophysiological measurement procedures.
Scheduled Sacrifice Time Points		Days 30 and 90

Key Evaluations and Assessments:

- Moribundity/mortality health checks: Twice daily
- Incision site observations: Daily until healed
- Clinical observations: Daily for 14 days, then weekly
- Body weights: Weekly
- Modified Irwin test/functional observational battery: Days 30 and 90
- Ophthalmic examinations: Days 30 and 90
- Electrophysiological measurements (ABR and DPOAE): Days 30 and 90
- Clinical pathology (hematology and clinical chemistry): Days 30 and 90
- DNA biodistribution, *hOTOX* mRNA expression, & (b) (4) immune response analysis: Days 30 and 90
- Gross necropsy, organ weights, and histology: Days 30 and 90

Key Results:

- There were no DB-OTO effects on general health, clinical observations, body weight, ophthalmic exams, neurological assessments, or clinical pathology parameters. There was no antigen-specific immune activation of mouse splenocytes in response to peptides derived from the otoferlin protein or the DB-OTO vector in the (b) (4) assay.
- Measurable levels of DB-OTO vector DNA were present in plasma samples from 2/4 Group 5 animals on Day 30. No animals had measurable levels of vector DNA in plasma samples on Day 90. The applicant did not measure *hOTOX* mRNA levels in plasma.
- Measurable levels of DB-OTO vector DNA and *hOTOX* mRNA were found in all cochlea samples from Group 5 and 6 animals on Days 30 and 90.
- In Group 5, one or more animals had measurable levels of DB-OTO vector DNA in adrenal gland, brain, DRG, heart, kidney, liver, lungs, parotid lymph node, spinal cord, spleen, and testis. The highest average levels of DB-OTO vector DNA in non-cochlear tissues were measured in the DRGs, cervical spinal cord, and brain samples and were more than 6-fold higher than the average levels measured in all other non-cochlear tissue types. No ovary samples had measurable levels of vector DNA, and 1/4 animals had measurable levels of vector DNA in lymph node and testis.
- In Group 5 animals, measurable levels of *hOTOX* mRNA were found in a subset of brain (3/4), cervical DRG (2/2), and cervical spinal cord (2/2) samples. The average levels of expression in these non-otic neural tissues were 4.9- to 33.0-fold lower than the average expression levels measured in cochlea on Day 90. All samples isolated from non-neural tissue types (adrenal gland, heart, kidney, liver, lungs, parotid lymph node, spleen and testis) had no measurable levels of *hOTOX* mRNA expression.
- Over the 3-month period, DB-OTO showed improved ABR thresholds in Group 3 (9.2×10^{10} vg/ear) males and females on Day 30 and in Group 2 (1.3×10^{11} vg/ear) females on Day 90 compared to Group 1 control animals.
- Procedure-related neurologic findings (circling behavior) were observed across all groups at similar incidences: 7/20 vehicle controls, 7/19 animals in Group 3 (9.2×10^{10} vg/ear), and 5/19 animals in Group 2 (1.3×10^{11} vg/ear). These findings were attributed to the

PSCC surgical procedure given the lack of dose response and similar incidence in controls.

- There were procedure-related histologic findings across all groups, included varying types of inflammation, new bone formation in and around the PSCC, and remnants from the surgical procedure (i.e., possibly remnants of tissue glue or portions of the delivery canula) in the PSCC and associated soft tissue.
- There were non-adverse DB-OTO-related histologic findings of minimal to mild infiltrates of mononuclear cells within the perilymph of the cochlea. This finding was partially reversible by Day 90 in Group 2 (1.3×10^{11} vg/ear) and Group 3 (9.2×10^{10} vg/ear) animals and was notably less in overall incidence and severity in the Group 3 animals compared to the Group 2 animals.
- No DB-OTO-related macroscopic or microscopic histopathological findings were observed in non-otic tissues on either Day 30 or 90. The NOAEL for DB-OTO in this study was determined to be the highest dose level evaluated at 1.3×10^{11} vg/ear.

Reviewer Comments:

- *hOTOF mRNA expression, driven by the hair cell-specific Myo15 promoter, was restricted to neural tissues, demonstrating targeted transgene regulation despite broader vector DNA biodistribution. The absence of histopathological findings in tissues with detectable vector DNA or mRNA expression, combined with the lack of mRNA expression in reproductive tissues, mitigates concerns about off-target effects and supports the safety profile for clinical translation. However, the long-term consequences of low-level transgene expression in non-target neural tissues (brain, DRG, spinal cord) remain unknown and warrant continued monitoring in human subjects.*
- *Procedure-related findings were observed across all groups including controls. The use of PSCC administration in PND 14-16 mice differs from the intended clinical RWM approach, limiting the direct translatability of procedure-related findings to the clinical setting.*
- *The DB-OTO-related microscopic findings of minimal-to-mild mononuclear cell infiltration in the cochlear perilymph appear to be non-adverse. They were not associated with morphological changes to spiral ganglion neurons or hair cells and showed partial reversibility by Day 90, with notably lower incidence and severity at the lower dose level (9.2×10^{10} vg/ear). The NOAEL of 1.3×10^{11} vg/ear provides an adequate safety margin above the biologically active dose range (4×10^{10} to 9.2×10^{10} vg/ear) demonstrated in this and other mouse studies, supporting a favorable benefit-risk profile.*

Study #18

Report Number		OTOF-10091
Date Report Signed		20-Dec-2022
Title		A 7- and 27-Week Toxicity and Biodistribution Study Following a Single Intracochlear Injection of DB-OTO in Cynomolgus Monkeys
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To evaluate potential toxicity and biodistribution of DB-OTO when administered bilaterally as a single IC injection to cynomolgus monkeys through the RWM of each ear using the clinical surgical delivery method
Study Animals	Strain/Breed	Naïve from Cambodia
	Species	Cynomolgus macaque (<i>Macaca fascicularis</i>)
	Age	22 to 63 months old
	Body Weight	Males: 2.4 to 3.9 kg Females: 2.4 to 3.2
	#/sex/group	3/sex/group/time point
	Total #	37 animals (plus 2 alternatives)
Test Article(s)		DB-OTO (Lot No. B564TOXP1)
Control Article(s)		Diluent (0.001% poloxamer 188)
Route of Administration		Bilateral IC administration through the RWM
Description of the Surgical/Administration Procedure		A postauricular incision was created, and a mastoidectomy was performed to expose the facial recess and tympanic cavity using sterile saline irrigation. A small fenestration was created in the lateral semicircular canal, and a catheter was inserted approximately 3-5 mm through a perforation in the RWM. 0.060 mL of test article was injected at 0.9 mL/hr using a syringe pump, with the catheter left in place for 5 mins post-injection to equilibrate the perilymph. After catheter removal, the RWM opening and canal fenestration were sealed with soft tissue. The incision was closed in layers, and the animal underwent the same procedure on the contralateral side.
Study Groups and Dose Levels		Group 1: Vehicle control Group 2: DB-OTO 1.2×10^{12} vg/ear (2×10^{13} vg/mL) Group 3: DB-OTO 4.4×10^{12} vg/ear (7.3×10^{13} vg/mL)
Dosing Regimen		Single bilateral administration
Randomization		Yes
Description of Masking		Data collection for ABR and DPOAE was conducted by a blinded technician.
Scheduled Sacrifice Time Points		Weeks 7 and 27

Key Evaluations and Assessments:

- Mortality, cage side observations, and qualitative food consumption: Daily
- Detailed clinical observations and body weights: Weekly
- Ophthalmology and neurological examinations: Once pretreatment and during Weeks 7 and 26
- Otoscopy: Once pretreatment and during Weeks 2, 5, 7, 11, 15, 19, 23, and 26
- Auditory observations (ABR and DPOAE): Once pretreatment and during Weeks 7 and 27

- Clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis): Twice pretreatment and Weeks 7 and 27
- DNA biodistribution (CSF, plasma, and selected tissues):
 - CSF: Day 2, Week 7/8, and 27
 - Plasma: Days 1, 2, 3, 5, 12, and 26; and Week 7, 12, 16, 20, and 27
 - Tissues: Week 7 and 27
- *hOTO*F mRNA expression (temporal bone tissue and any non-otic tissues positive for vector DNA): Week 7 and 27
- Neutralizing antibodies (NAbs) (CSF and serum):
 - CSF: Day 2, Week 7/8, and 27
 - Serum: Once pretreatment and Week 7 and 27
- Anti-otoferlin antibodies (CSF and serum):
 - CSF: Day 2, Week 7/8, and 27
 - Serum: Once pretreatment and Week 4, 7, and 27
- Viral shedding (buccal mucosal swabs, nasal swabs, feces, and urine): Day 2, Week 7, and Week 26
- PBMC (peripheral blood mononuclear cell) (b) (4) analysis for IFN- γ : Once pretreatment and Week 7 and 27
- Organ weights and macroscopic and microscopic pathology examinations (including a specialized assessment of step sections through the cochlea for ototoxicity and peer review): Week 7 and 27

Key Results:

- There were no DB-OTO related effects on mortality, clinical observations, qualitative food consumption, body weight, neurologic exams, otoscopy exams, ABR, DPOAE, (b) (4), hematology, coagulation, clinical chemistry, urinalysis, organ weights, macroscopic or microscopic histopathology, or ototoxic histopathology.
- Procedure-related clinical observations were observed across all groups, including controls, and consisted of acute, transient effects (Days 1-5) including vomiting, decreased activity, lack of coordination, tremors, nystagmus, ataxia, head tilt, dry eye, and hunched posture. Additional observations included transient body weight loss (recovered by Day 21) and surgical site complications such as abrasions, lacerations, and infections requiring surgical repair through Day 44.
- Facial nerve paralysis occurred in 6/36 animals (3/12 controls and 3/24 DB-OTO-administered animals). Of the DB-OTO-administered animals that developed facial paralysis, 2 showed complete recovery by Day 3, while 1 animal administered 1.2×10^{12} vg/ear was euthanized on Day 3 due to procedure-related unilateral facial paralysis, corneal abrasions, and limited treatment options in the nonclinical setting. The 3 control animals with facial paralysis did not fully recover prior to their Week 7 necropsy, with 1/3 animals showing decreased palpebral and menace reflex on neurological examination.
- Procedure-related ABR threshold elevations were observed at similar incidences across all groups including controls, affecting 55-84% of ears at higher frequencies (8-32 kHz) and 21-33% of ears at lower frequencies (1-4 kHz) important for speech perception. ABR thresholds showed subtle signs of recovery between Week 7 and Week 27 at higher

frequencies, while DPOAE responses tended to worsen slightly over this period. The distribution of ABR changes remained stable between the two time points. Pronounced ABR threshold elevations were generally associated with more severe histopathological findings, but the overall hearing impacts appeared to be artifacts of the surgical intervention as they occurred across all DB-OTO and control groups.

- DB-OTO vector DNA was detected in temporal bone samples from all DB-OTO-administered animals (except one Group 3 animal) at both Week 7 and Week 27, with mean concentrations increasing with dose level and remaining sustained over the 27-week study duration. Vector DNA concentrations in plasma peaked at 8 hours post-dose and decreased rapidly, with negligible levels by Week 7 (only one animal had DB-OTO-3 vg at 1 copy above the lower limit of quantification (LLOQ)). In CSF, measurable levels were detected in 3/12 animals at the high dose level (4.4×10^{12} vg/ear) at 24 hours post-dose, with no detectable levels by Week 7/8.
- Systemic distribution of DB-OTO vector DNA was limited. At Week 7, vector DNA was detected in multiple non-otic tissues including auditory nerve, parotid lymph node, brain, DRG, spinal cord, heart, kidney, lung, liver, and spleen. Vector DNA levels in these tissues were at least 10-fold lower than temporal bone levels, with the exception of the auditory nerve, which showed variable levels including individual animals with high vector concentrations. By Week 27, fewer tissue types had measurable vector DNA, with reduced detection frequency in most tissues. Vector distribution to reproductive tissues was negligible, with no measurable levels in testes and only one ovary sample at Week 7 with low levels near the LLOQ (no detection at Week 27).
- All temporal bone samples from DB-OTO-administered animals at both Week 7 and Week 27 showed measurable *hOTOF* mRNA levels with considerable inter-animal variability. Outside the temporal bone, *hOTOF* mRNA expression was restricted to neural tissues, detected only in cervical and lumbar DRG samples from one Group 3 animal at Week 7, with no measurable expression in any non-otic tissues at Week 27. No *hOTOF* mRNA was detected in reproductive tissues or other non-neural tissues.
- Group 2 animals (1.2×10^{12} vg/ear) showed higher average temporal bone *hOTOF* mRNA expression levels than Group 3 animals (4.4×10^{12} vg/ear) at both time points despite receiving a lower dose level. However, this difference was not statistically significant due to high inter-animal variability.
- Animals with positive and negative screening of anti-AAV1 NABs at baseline were included in the study. At Week 27, all animals administered 1.2×10^{12} and 4.4×10^{12} vg/ear DB-OTO were positive for anti-AAV1 NABs in serum with titers generally equal to or increased relative to the Week 7 time point. One animal administered DB-OTO at 1.2×10^{12} vg/ear had a positive titer for NABs in CSF at Week 27 (1:50). There were no serum or CSF samples positive for anti-otoferlin antibodies at any time point. Pre-existing or post-DB-OTO administration positive serum or CSF anti-AAV1 NAB titers were not associated with any safety signal. There was no clear correlation observed between serum or CSF NAB titers and the resulting levels of vector DNA or *hOTOF* mRNA in the cochlea.
- In all 4 tested shedding matrix types (buccal mucosal swabs, nasal swabs, feces, and urine), measurable levels of DB-OTO vector DNA were found on Day 2 in one or more samples from animals administered 4.4×10^{12} vg/ear. No DB-OTO vector DNA was detected in any of the shedding samples collected at the Week 7 time point.

- Procedure-related microscopic changes in the middle and inner ear were observed at similar incidences and severities across all groups including controls. Middle ear findings included granulation tissue formation, mononuclear cell infiltration and hyperplasia of the mucoperiosteum, adhesions between middle ear structures, and new bone formation at the lateral semicircular canal fenestration site that maintained patency in all evaluable animals. Inner ear findings included spiral ganglion cell degeneration (2/6 control animals and 7/12 DB-OTO-administered animals at Week 27), decreased cellularity of hair cells in the organ of Corti, degeneration of the osseous spiral lamina, and decreased cellularity of the spiral limbus. These degenerative changes were primarily localized to the basal turn of the cochlea near the RWM injection site in most animals, though one DB-OTO-administered animal experienced marked damage extending beyond the basal turn with marked axonal degeneration of the auditory nerve. Mononuclear cell infiltration of the scala tympani/vestibuli showed significant recovery (9/18 animals at Week 7 to 1/18 animals at Week 27 across all groups), while degenerative changes tended to show progression in incidence and/or severity at Week 27.

Reviewer Comments:

- *DB-OTO demonstrated an acceptable safety profile with no DB-OTO-related adverse effects. All observed safety findings were attributed to the surgical procedure rather than the test article, with the NOAEL established at the highest dose level evaluated of 4.4×10^{12} vg/ear (concentration of 7.3×10^{13} vg/mL). This safety profile supports the clinical use of DB-OTO, as the absence of DB-OTO-related toxicity at the highest dose level evaluated provides an adequate safety margin for therapeutic administration. The clinical dose level is 7.2×10^{12} vg/ear (concentration of 3×10^{13} vg/mL based on 0.24 mL human perilymph volume), representing a safety margin of approximately 0.6-fold by total dose level (vg/ear) and 2.4-fold by concentration (vg/mL). Since DB-OTO is delivered locally to replace the perilymph volume, dose scaling by concentration is considered the more relevant metric for safety assessment. See discussion under 'Nonclinical Rationale for Clinical Dose Level Selection.'*
- *This study provides the most clinically relevant procedural safety data, as it utilized the same RWM surgical approach intended for clinical use and was performed in monkeys with inner ear anatomy similar to humans. Procedure-related complications were observed across all groups including controls, with findings of particular concern including incomplete recovery from facial paralysis in some animals and degenerative changes in spiral ganglion cells and hair cells associated with permanent auditory damage. The clinical relevance of these findings remains uncertain, as the cynomolgus monkey model may not fully predict the human surgical experience due to minor anatomical differences, potential surgical technique refinements in the clinical setting, and the fact that the target subject population already has profound hearing loss. Thus, clinical monitoring will be important to characterize the procedural safety profile in human subjects with OTOF-related hearing loss, with particular attention to facial nerve function and hearing outcomes at speech-relevant frequencies.*

- *DB-OTO showed effective local retention with minimal systemic exposure. Vector DNA was sustained in temporal bone samples through Week 27, while systemic distribution was transient with rapid clearance from plasma and CSF, and viral shedding was only detected on Day 2. This biodistribution profile supports the local therapeutic activity of DB-OTO in the target cochlear tissue, while minimizing potential systemic safety concerns from off-target exposure.*

Nonclinical Modeling Studies

Study Number	Study Title	Report Number
19	Nonclinical Modeling to Support DB-OTO First-in-Human Dose Selection	OTOF-10116-SR-01V1

Study #19

Nonclinical Modeling to Support DB-OTO First-in-Human Dose Selection

Objective:

To conduct a pharmacodynamic modeling study using nonclinical data to characterize the dose-response relationship between DB-OTO administration and auditory function improvement, with the goal of supporting the clinical dose selection.

Methods:

The study utilized a nonlinear, mixed-effects model to analyze ABR threshold data from three pharmacology studies in mice: DB-OTO-PHAR-017, DB-OTO-PHAR-023, and OTOF-10104. The model incorporated data from both DB-OTO-administered and uninjected Otof-Q828X hom mice, as well as uninjected Otof-wt mice, across all studied dose levels. Data from mice with severe procedure-related hearing loss (as indicated by DPOAE loss) were excluded from the analysis.

Results:

The model identified an Emax (maximum effect) relationship between the DB-OTO dose concentration and the improvement in ABR thresholds. Threshold improvements approached a plateau at dose concentrations greater than 2×10^{13} vg/mL, which corresponds to a dose level of 4×10^{10} vg/ear in mice. At the maximum feasible dose concentration, the model predicted that ABR threshold improvements in DB-OTO-administered mutant mice would approach within 11 to 19 dB of the thresholds observed in normal-hearing Otof-wt mice.

Conclusion:

The modeling analysis demonstrated that a dose concentration of 2×10^{13} vg/mL results in a substantial and near-maximal therapeutic effect on hearing function in the nonclinical model. Based on these findings, the proposed clinical dose concentration of 3×10^{13} vg/mL, which is 1.5-fold higher than the concentration where substantial benefit was observed, is expected to be on the efficacy plateau and has the potential to produce a clinically meaningful improvement in hearing for subjects with *OTOF*-related deafness.

Nonclinical Rationale for Clinical Dose Level Selection:

The selection of the proposed clinical dose concentration of 3×10^{13} vg/mL is supported by the pharmacodynamic modeling (OTOF-10116) of ABR improvements in the Otof-Q828X hom mouse model as well as the safety profile established in the GLP toxicology studies. The pharmacodynamic modeling identified that the therapeutic effect approached a plateau at dose concentrations greater than 2×10^{13} vg/mL (corresponding to an MED of 4×10^{10} vg/ear). In the toxicology studies, the NOAELs were identified as the highest concentrations evaluated (6.5×10^{13} vg/mL in mice and 7.3×10^{13} vg/mL in monkeys), which provides safety margins of approximately 2.2-fold and 2.4-fold, respectively. This cross-species dose rationale is based on achieving an equivalent concentration (vg/mL) in the inner ear, as DB-OTO is administered locally to replace the full volume of perilymph. Collectively, the nonclinical data suggest that the proposed clinical dose level has a reasonable expectation to provide a durable therapeutic effect while maintaining a favorable safety profile.

APPLICANT'S PROPOSED LABEL

Section 12.3 '*Pharmacokinetics*' should be revised to accurately reflect the available nonclinical data. Section 13 '*Nonclinical Toxicology*' should be revised to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

OTARMENI™, lunsotogene parvec cwha, DB-OTO, otoferlin, OTOF, hOTOF, deafness, hearing loss, intracochlear, auditory brainstem response, ABR, distortion product otoacoustic emissions, DPOAE, adeno-associated vector, AAV1, inner hair cells, outer hair cells, cochlea, perilymph, cochlear implant, deafness, round window membrane, RWM

REFERENCES

1. Antia SD, Lederberg AR, Easterbrooks S, Schick B, Branum-Martin L, Connor CM, et al. Language and Reading Progress of Young Deaf and Hard-of-Hearing Children. *J Deaf Stud Deaf Educ.* 2020;25(3):334-50.
2. Ford CL, Riggs WJ, Quigley T, Keifer OP, Jr., Whitton JP, Valayannopoulos V. The natural history, clinical outcomes, and genotype-phenotype relationship of otoferlin-related hearing loss: a systematic, quantitative literature review. *Hum Genet.* 2023;142(10):1429-49.

3. Kronenberger WG, Pisoni DB, Henning SC, Colson BG. Executive functioning skills in long-term users of cochlear implants: a case control study. *J Pediatr Psychol*. 2013;38(8):902-14.
4. Marschark M, Shaver DM, Nagle KM, Newman LA. Predicting the Academic Achievement of Deaf and Hard-of-Hearing Students from Individual, Household, Communication, and Educational Factors. *Except Child*. 2015;81(3):350-69.
5. Martin J, Hamilton B, Osterman M. Births in the United States, 2023. NCHS Data Brief, no 507. Hyattsville, MD: *National Center for Health Statistics*; 2024. Available from: <https://www.cdc.gov/nchs/data/databriefs/db507.pdf>
6. Morton CC, Nance WE. Newborn hearing screening--a silent revolution. *The New England journal of medicine*. 2006;354(20):2151-64.
7. Pangrsic T, Reisinger E, Moser T. Otoferlin: a multi-C2 domain protein essential for hearing. *Trends Neurosci*. 2012;35(11):671-80.
8. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL. Language of early- and later-identified children with hearing loss. *Pediatrics*. 1998;102(5):1161-71.