Title: Fibroblast Growth Factor Regeneration of Tympanic Membrane Perforations

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TITLE PAGE

Study Title: Fibroblast Growth Factor Regeneration of Tympanic Membrane Perforations

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Principal Investigator: D. Bradley Welling, M.D., Ph.D.

IND Number: 130892

Study Phase: Phase II

Study Product: Fibroblast Growth Factor-2 (bFGF/FGF-II)

1. SYNOPSIS

Protocol No.: 16-043H

TITLE: Fibroblast Growth Factor Regeneration of Tympanic Membrane Perforations
INVESTIGATIONAL PRODUCT: Human fibroblast growth factor-2 (FGF-2/bFGF/FGF-II) in a gelatin sponge, adhered by fibrin glue
INDICATION: Treatment of chronic non-healing tympanic membrane perforation
PHASE OF DEVELOPMENT: Phase 2
INVESTIGATIONAL SITES/LOCATIONS: Mass Eye and Ear Infirmary, Boston, Massachusetts
STUDY DESIGN: A Phase II randomized trial will be initiated to evaluate efficacy as the primary measureable outcome. Eligible and consented patients will be randomized in a 3:1 ratio to the optimal biologic dose (OBD) of FGF-2 or placebo (sterile water) during the Randomized Treatment Phase. Application of the study treatment may occur at the initial Screening Visit (pending all eligibility requirements can be confirmed) or at Visit 1 and may be repeated at each follow up visit as needed for a maximum of three treatments in the Randomized Treatment Phase. These follow up visits will occur three weeks (+/- 7 days) from the last treatment application. During these visits, the subject's tympanic membrane will be examined, photographed, and additional otologic tests will be performed. If the perforation is found to be closed during these visits, then the subject will report in 2 months (+/-7 days) for a final study visit. If the subject's TMP has not closed after the third and final application of their assigned treatment group, then the blind on the subject's randomization status will be broken, and placebo patients will be crossed over to receive unblinded study drug. Again, the subject may be given up to three additional treatments in the unblinded crossover phase. The follow up visits will follow the same schedule, occurring three weeks (+/- 7 days) from the last treatment. The same otologic procedures will be performed as in the previous visits. All subjects will report for a final study visit 2 months (+/- 7 days) after their last follow up visit when the perforation is determined to be closed or when the subject has completed the maximum number of study visits without perforation closure. The study will be completed for reporting purposes after all subjects have completed the 2 month (+/- 7 days) follow up final visit.
NUMBER OF SUBJECTS (PLANNED): 75

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Key inclusion criteria:

- Age 18 years and older
- Dry tympanic membrane perforation of greater than 3 months duration
- If female, post-menopausal, sterile, or if she is of child-bearing potential, she must have a negative beta-HCG test and must agree to use an adequate form of birth control throughout the clinical trial.

Key exclusion criteria:

- Active otitis media or chronic otorrhea from the middle ear
- Subjects receiving radiation therapy, corticosteroids, immunosuppressive agents, or chemotherapy
- Subjects who, at study entry, are taking systemic antibiotics
- Subjects who are immunosuppressed
- Subjects experiencing bacterial or viral infection or who may otherwise be febrile
- Life expectancy of less than 1 year
- Active alcohol or drug abuse within 6 months prior to study entry
- Significant medical condition that could prevent full participation in the procedures required for the study (see Section 7.2 for full list of exclusions)
- Known or suspected allergies to any components used in the study, i.e. porcine collagen
- Subjects who have cholesteatoma mass in the tympanic cavity
- Subjects whose total perforation cannot be seen by an endoscope
- Subjects with inadequately controlled diabetes mellitus (NGSP: HbA1c 6.9% or higher)
- Subjects with a history of malignant ear canal tumors within 3 years of screening for eligibility

TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION:

Subjects will receive an estimated 0.2 mL of 100 μg/mL of FGF-2. The FGF-2 will be mixed from 250 μg (300,000 international units) of lyophilized FGF-2 with 2.5 ml of sterile water. After saturation of the gelatin sponge, it is estimated that the gelatin sponge will contain approximately 20 μg in 0.2 ml of the solution. The actual size of the pledget may vary slightly depending upon the size and configuration of the perforation. The application of the FGF-2 will proceed according to the following protocol. The fibrin glue consists of human fibrinogen, human blood coagulation factor XIII, aprotinin solution, thrombin, and calcium chloride hydrate.

1) In an outpatient examination, confirm that the patient has a tympanic membrane perforation and no active infection/inflammation is present, then insert a cotton ball soaked in 2% lidocaine jelly directly to the surface of the tympanic membrane under the microscopic visualization.

2) After 15 minutes, the lidocaine infused cotton ball will be removed and the edge of the perforation roughened with a micropick to remove the epithelium circumferentially around the perforation edge.

3) Place a human fibroblast growth factor-2 (FGF-2) soaked pledget of bioabsorbable gelatin sponge larger than the tympanic membrane so the tympanic membrane is completely covered.

4) Secure the pledget in place with several drops of fibrin glue.

5) After the effects of the topical anesthetic have worn off, if any, the patient is released with the following instructions:

a) Avoid violently sniffling or blowing the nose
b) Sneeze and cough naturally through the mouth without occluding the mouth with the hand.
c) Use cotton balls covered with petroleum jelly on the outer surface when washing the hair and bathing. This is needed to help keep water out of the ear.
d) Avoid using a hearing aid in the treated ear.
e) Avoid flying, using elevators in tall buildings, and other situations involving substantial changes in air pressure.
f) Avoid swimming.

DISCONTINUATION FROM TREATMENT:

Reasons for permanent discontinuation include the following:

If the first treatment does not result in complete closure, subsequent treatments will not be administered if any ear complications occur in the treated ear, including but not limited to infection, moderate to severe pain, moderate to severe irritation, or clinically significant sensorineural hearing loss. All subjects will complete all safety follow up visits.

PRIMARY ENDPOINT:

The tympanic membrane and surrounding structures will be examined every 21-days (+7 days) for up to three visits. Adverse events will be recorded at all visits. Routine laboratory tests will be performed at the screening visit and at the final study visit. Vital signs will be assessed at each study visit and changes in clinical examination findings will be noted.

SECONDARY ENDPOINTS:

- Tympanic membrane perforation closure determined by otoscopic exam and photographic documentation
- Pure-tone and speech discrimination scores as measured by pre- and post-treatment audiograms
- Mobility of the tympanic membrane as measured by tympanometry
- Salvage rate of FGF-2 in patients who fail placebo

STATISTICAL ANALYSIS:

The target enrollment of 75 subjects will provide sufficient power while also accounting for potential retention issues. The primary endpoint is to confirm the efficacy of

FGF-2 in subject with TMR by testing the hypothesis that the rate of subject with complete closure of the TMR will be superior to that placebo.

The sample size is calculated in 3:1 ratio to FGF-2 treatment group or placebo to provide more than 90% power to detect a clinical significant difference at a two-sided significance level of 0.05 using a Fisher's exact test. To allow for 5% dropouts, 45 patients of FGF-2 treatment group and 15 patients of placebo are required.

Data after discontinuations for any reason will not be imputed through the final schedule visit. This subject will be considered as not-improvement.

1.2 Terms, Acronyms, Abbreviations

Term	*Definition*
AA	Amino acid
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BP	Blood pressure
°C	Degree Celsius
CBC	Complete blood count
CFR	Code of Federal Regulations
CRF	Case Report Form
CRA	Clinical Research Associate
dB	Decibels
DCF	Data Collection Form
DTF	Data Transmittal Form
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EDC	Electronic data capture
EGF	Epidermal growth factor

Term	*Definition*
FDA	Food and Drug Administration (U.S.)
bFGF/FGF-2	Basic fibroblast growth factor-2
GCP	Good Clinical Practices
HCG	Human chorionic gonadotropin
h(s)	Hour(s)
HS	Heparin sulfate
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IRB	Institutional Review Board
kDa	Kilodalton
LLN	Lower limit of normal
mL	Milliliters
mm	Millimeters
MTD	Maximum tolerable dose
N/A	Not applicable
NCICTC	National Cancer Institute Common Toxicity Criteria
NLS	Nuclear localization sequence
NS	Not significant
NYHA	New York Heart Association
OBD	Optimal biologic dose
PBS	Phosphate buffered saline
PDF	Portable document format
PE	Physical examination

Term	*Definition*
PP	Per protocol
SAE	Serious Adverse Event
SPL	Sound pressure level
T	Temperature
TBD	To be determined
TM	Tympanic membrane
TMP	Tympanic membrane perforation
t-PA	Tissue plasminogen activator
ULN	Upper limit of normal
USP	United States Pharmacopeia
VT	Ventilation tubes
WBC	White blood cell

2. Introduction

The tympanic membrane, also called the eardrum, is a flexible, translucent, diaphragm like structure. Tympanic membrane perforations (TMP) can result from disease, trauma, or medical care. Perforations can be temporary or persistent. The effect of TMP varies with size, location on the drum surface, and associated pathologic conditions. Perforation symptoms include audible whistling sounds during sneezing and nose blowing, decreased hearing, especially with larger perforations, and a tendency to infection during upper respiratory infections and when water enters the ear canal.

Up to 50 percent of TMPs are refractory to spontaneous healing and require surgical repair depending upon the etiology of the perforation. There is a higher incidence of middle ear contamination with resultant increased incidence of acute and chronic otitis media and chronic middle ear scarring or tympanosclerosis with a resultant conductive hearing loss. Profound sensorineural hearing loss, balance dysfunction, meningitis or brain abscess are rare but serious complications, which may occur from middle ear infection. Long-term effects may include speech and language acquisition delay in children as well.

Some subjects undergo multiple attempts at surgical repair without attaining closure of the tympanic membrane. The underlying cause of the perforation may be an important factor in the inability of the body to spontaneously close the perforation or the surgeon's inability to get engraftment and surgical closure. For example, traumatic perforations of the tympanic membrane generally, close spontaneously within three months of the injury. Perforations caused by hot slag burns in welders are notoriously difficult to get to heal even with surgical intervention. Blast injuries from improvised explosive devices spontaneously heal in less than 50% of cases. The latter two conditions may be due to poor vascularity of the cauterized remnant TM and damaged external auditory canal skin.

Other etiologies such as acute otitis media, poor eustachian tube function, or retained tympanostomy tubes can predispose to chronic perforations as well. Ingrowth of squamous epithelium from the outer layer of the TM around the edge into the middle ear through the perforation preventing fibroblast proliferation and contraction of this wound may prevent spontaneous healing also. While small perforations are not usually associated with hearing loss, large perforations can be associated with a significant conductive hearing loss (up to 30 dBSPL).

Several surgical techniques are available for repair of chronic perforations. A myringoplasty involves placement of a paper patch, plug of fat, or gelatin sponge over the surface or in a smaller perforation (<4 mm). This procedure provides a scaffold for fibroblast migration and contraction of the perforation. Myringoplasty can be of some benefit for small perforations but is unlikely to be successful for large perforations. Tympanoplasty uses the patients native fascia or perichondrium as a scaffold placed either under the remaining tympanic membrane or over the surface of the remnant drum (medial and lateral graft tympanoplasty respectively). Both surgical procedures are usually performed in an outpatient operating room setting and require anesthesia.

FGF has been shown to stimulate the division and differentiation of fibroblasts and promote angiogenesis thereby improving blood flow and healing. Mondain and Ryan (1993) found expression of bFGF three days after a traumatic perforation, mainly in the perforated area, indicating involvement of bFGF in TM healing. They demonstrated acceleration of healing of TM perforations after a single application of bFGF in a rat model at dose levels of 0.4 and 2 μg (0.01–0.05 mg bFGF/ml). They found hyperplasia of the connective tissue, with naturally-oriented fibroblasts in the lamina propria of the bFGF-treated ears.

2.1 Primary Disease Information

2.2 Natural History of TM Perforation

Chronic TM perforation is a recognized complication of ventilation tube (VT, grommet) placement, and several papers have reported such iatrogenic perforations, defined expected incidence, and investigated various strategies for prevention. Few studies have investigated the natural history of TM abnormalities in general. One study followed a general population cohort of 358 children, with a standardized examination at 8 and 18 years and showed a favorable prognosis for TM abnormalities (tympanosclerosis, atrophy, atelectasis, retraction, and perforation) with conservative management during the 10-year study period, although in this data set, perforations were quite unusual.

A meta-analysis in 2001 calculated a post-VT perforation rate of 4.8% with all types of VT design included (964/20,222 ears from 62 studies), 2.2% with VTs designed for short-term use (175/8107 ears from 29 studies), and 16.6% with VTs designed for long-term use (556/3356 ears

from 21 studies). A single investigation of 2604 VT insertions showed a rate of persistent perforations of 3.1%.

A study in 1996 examined 57 perforated membranes in 47 subjects and showed no particular difference in size and site of perforation, or level of conductive hearing loss, between 41 VT-related and 16 non-VT- related perforations. Other investigations have centered on traumatic perforations. Several articles serve to confirm the widely held belief that early presentation and correct management improve outcomes, and articles investigating time to closure following perforation showed that spontaneous closure is unlikely if not complete in 2.5 years (Rollin et al, 2011).

2.3 Study Drug Information

The name "fibroblast growth factor" (FGF) is a limited description for this family of cytokines. The function of FGFs is not restricted to fibroblast cell growth. Although some of the FGFs do, indeed, induce fibroblast proliferation, the FGF molecule (FGF-2 or FGF basic) is now known to also induce proliferation of endothelial cells, chondrocytes, smooth muscle cells, and melanoacytes. It can also promote adipocyte differentiation, induce macrophage and fibroblast IL-6 production, stimulate astrocyte migration, and prolong neuronal survival. Thus, the FGF designation is clearly limited by its description of one target cell and one implied biological activity. To date, the FGF superfamily consists of 22 members, all of which contain a conserved 120 amino acid (AA) core region that contains six identical, interspersed amino acids. The superfamily members act extracellularly through four-tyrosine kinase G-coupled protein receptors, with multiple specificities noted for almost all FGFs. This likely accounts for similar effects generated by many FGF molecules on common cell types. The FGFs are now known to play substantial roles in development, angiogenesis, hematopoiesis, and tumorigenesis.

2.4 Human FGF Structural Information

The 22 human FGFs identified include FGF-1 through 14 plus 16 through 23. In general, their native molecular weights range from 7 kDa (FGF-1) to 38 kDa (FGF-5), and their AA lengths vary from 60 AA (an FGF-1 splice variant) to 288 AA (FGF-2). These AA lengths are generally determined by three coding exons. Over coding regions beginning with an AUG (methionine) start site, pairwise AA identity ranges from 17% to 72% (20-30% on average), with seven human subfamilies identified based on AA homology. These include FGFs-1 & 2, FGFs-3 & 5, FGFs-11,

12, 13 & 14, FGFs-4 & 6, FGFs-9, 16 & 20, FGFs-7 & 10, and FGFs-8, 17 & 18; human FGF-19 is closest to mouse FGF-15, and human FGF-21 has no counterpart. In addition to AUG start sites, many FGFs utilize CUG (leucine) start sites, and this adds a 5' AA extension to many molecules. Further complexity is introduced by the fact that select FGFs (FGF-8 and -17) exhibit a division of the first exon into three or four mini-exons, creating the possibility for multiple N-terminal splice forms. Although there is only very modest AA identity between all 22 human family members, the one characteristic apparently shared among all members is the three-dimensional, ß-trefoil motif (*i.e.*, a fold which consists of 12 anti-parallel ß-strands). This configuration may prove to be critical for receptor recognition. There is considerable FGF AA identity across species, and species cross-reactivity is common among FGF superfamily members.

Human FGF-2, otherwise known as FGF basic, HBGF-2, and EDGF, is an 18 kDa, non-glycosylated polypeptide that shows both intracellular and extracellular activity. Like FGF-1, the 18 kDa molecule is 155 AA in length when translated from an AUG (methionine) start codon. Unlike FGF-1, there are at least four alternate CUG (leucine) start codons that provide N-terminal extensions of 41, 46, 55, or 133 AA, resulting in proteins of 22 kDa (196 AA), 22.5 kDa (201 AA), 24 kDa (210 AA) and 34 kDa (288 AA), respectively. Although these extensions could be assumed to impart special activities to the higher molecular weight (HMW) forms, all FGF-2 forms have the potential to perform the same function. The fact that they do not seems more related to their anatomical location than their structure. Differences noted for 18 kDa *vs.* 24 kDa FGF-2 include an up-regulation of tissue plasminogen activator (t-PA) for 18 kDa FGF-2 and a down-regulation of t-PA for 24 kDa in pancreatic carcinoma cells, and an increase in IL-6 expression for 24 kDa FGF-2 contrasted with a decrease in IL-6 expression for 18 kDa/FGF-2 in 3T3 fibroblasts. In general, the 155 AA/18 kDa form is considered cytoplasmic (secretable), while the high molecular weight (HMW) forms are considered nuclear in target. Although less is known about the secretion of FGF-2 than FGF-1, release of the 18 kDa form is reported to be an energy-dependent process that is independent of the ER-Golgi. Unlike FGF-1, FGF-2 is secreted as a monomer. Following secretion, FGF-2 is sequestered on either cell surface HS or matrix glycosaminoglycans. Although FGF-2 is secreted as a monomer, cell surface HS seems to dimerize monomeric FGF-2 in a non-covalent side-to-side configuration that is subsequently capable of dimerizing and activating FGF receptors. Notably, FGF-2 responsive cells may be able to block cell surface dimerization of basic FGF by ribosylating 18 kDa FGF-2 in the receptor-binding domain (AA 106-120). In addition to

inducing FGF receptor signal transduction, bound 18 kDa FGF-2 can be internalized, either in association with HS or its receptor. Once internalized, FGF-2 can be degraded, possibly into 4-10 kDa bioactive fragments, or translocated to the nucleus as a receptor-ligand complex. HMW forms of FGF-2 are associated with nuclear translocation. Although there is an nuclear localizing sequence (NLS) in the N-terminus of all HMW forms, it has been suggested that methylation of arginines 24-28 AA upstream of the first methionine of 18 kDa FGF-2 is responsible for nuclear entry. Entry into the nucleus is associated with FGF-induced effects such as casein kinase II activation, which is necessary for cell-cycle progression and proliferation. Human 18 kDa FGF-2 is 97% AA identical to both mouse and rat FGF-2. Cells known to express FGF-2 include visceral and vascular smooth muscle cells, cardiac muscle cells, lining epithelium of the colon and bronchus, neurons, plus cerebellar Purkinjie cells, megakaryocytes and platelets, endothelial cells, mast cells, glomerular parietal epithelial cells and podocytes, astrocytes, $CD4^{+}$ and CD8 T cells, fibroblasts (plus extracellular matrix), and numerous embryonic mesodermal and neuroectodermal tissues.

3. Previous Human/Animal Experience

3.1 Nonclinical Use of FGF in Experimental TMR

Investigators have examined growth factor expression and treatment in chronic perforations in animal models. Epidermal growth factor in our early studies showed complete TMP closure in a chinchilla model, however, epithelial ingrowth forming cholesteatomas was found in 18% of animals. (Dvorak et al, 1995).

FGF has been shown to stimulate the division and differentiation of fibroblasts and promote angiogenesis thereby improving blood flow and healing. Mondain and Ryan (1995) found expression of bFGF three days after a traumatic perforation, mainly in the perforated area, indicating involvement of bFGF in TM healing. They demonstrated acceleration of healing of TM perforations after a single application of bFGF (Mondain and Ryan 1994, Mondain et al., 1991) in a rat model at dose levels of 0.4 and 2 µg (0.01–0.05 mg bFGF/ml). They found hyperplasia of the connective tissue, with naturally-oriented fibroblasts in the lamina propria of the bFGF-treated ears.

Using a similar model, Vrabec et al. (1994) investigated a multiple dosing regimen of 5 µg bFGF (0.1 mg bFGF/ml) and also found an accelerated rate of healing in the bFGF-treated ears.

Fina et al.[11] used a multiple dosing regimen of 1 μg bFGF (0.2 mg bFGF/ml) combined with a gelfoam scaffold in acute perforations in guinea pigs and found a more rapid rate of healing of the TM in comparison with that in placebo-treated ears. In a follow-up study, these investigators demonstrated more rapid healing of acute perforations with the use of bFGF solution alone, without using a gelfoam sponge to serve as a reservoir and mechanical scaffold (Fina et al., 1993)[7]. Kato and Jackler (1996)[8] investigated the effects of bFGF with gelatin sponge carrier on the healing of TM perforations. bFGF (5 μg) in 25 ml of PBS (0.2 mg bFGF/ml) was administered to a gelatin sponge pledget placed over chronic TM perforations in chinchillas and they found an 81% closure rate compared to 41% in the control group.

No apparent risks of ototoxicity were observed in Kase et al's 2007 study of two groups of 21 healthy guinea pigs, in which 100μg of b-FGF was applied four times to the left external auditory canal and middle ear using a gelatin sponge container (Spongel; Astellas Pharma Inc, Tokyo, Japan) for Group A (n=10) or myringotomy and then a gelatin sponge insertion into the middle ear for Group B (n=11). The right ears served as the controls. The cochleae were examined one week post application in addition to measuring endocochlear DC potential (EP). Topical application of the drug did not cause significant reduction in EP or any degenerative changes in the cochlea.

A more recent study conducted by Hakuba et al (2014) demonstrated that a sustained b-FGF formulation could be applied to and successfully close eardrum perforations in guinea pigs using a gelatin hydrogel. In this equivalent 3-group model of 24 guinea pigs (b-FGF-gelatin hydrogel group, saline gelatin hydrogel group, and control group), total tympanic membrane perforations were formed using a CO_2 laser. All 8 (100%) TMPs in the b-FGF gelatin hydrogel group achieved closure of the perforation, compared to 3 out of the 8 ears in the saline-gelatin group (37.%) and none of the control group (0%). In addition to improving closure rates, the application of b-FGF also induced improved regeneration of the fibrous layer in addition to the mucosal and epithelial layers. Overall, this study showed promising results in terms of *in vitro* and *in vivo* benefits. *In vitro*, there was fibroblast growth as well as migration and growth, via FGF receptors, of various cells involved in wound healing (including vascular endothelial cells, vascular smooth muscle cells, and epithelial cells). Application of b-FGF also promoted neovascularization and granulation tissue formation.

3.2 Clinical Use of FGF-2

A study in 87 subjects with chronic tympanic membrane perforation was recently published. The objectives of the study was to measure the rate of complete closure of chronic TMR using basic fibroblast growth factor (bFGF) combined with an atelocollagen/silicone bilayer membrane patch. The study assessed closure of TMR in 87 subjects using bFGF, which is thought to promote the regeneration of TM tissues by facilitating the growth of fibroblasts and collagen fibers. A dose of 10 mcg of bFGF was administered every 21-days. Study methods included a procedure using an operating microscope. The results of the study reported mean perforation size before treatment of 14.4%. Complete closure of the TMR was achieved in 80 subjects (92.0%), whereas pinholes remained in 5 subjects (5.7%), and small perforations were observed in 2 subjects (2.3%). In the subjects with complete closure, the TMR closed after an average 1.8 treatments, and hearing improved by 13.6 dB. In conclusion the study demonstrated that bFGF combined with atelocollagen is effective for the conservative treatment of TMR (Hakuba et al, 2009).

Kanemaru et al (2011) have reported updated findings from their 2009 presentation involving 63 subjects, in which 53 received tympanic membrane repair using gelatin sponge and fibrin glue with b-FGF and 10 were assigned to the control group of gelatin sponge and fibrin glue alone. The study sought to determine closure rates, hearing level, and sequelae after 3 weeks post treatment. Of the 53 subjects who received b-FGF treatment, 52 had a complete closure of the TMP (98.1%) compared to 1 out of 10 (10%) of the control group subjects. Hearing level improved for all subjects with successful TM repair. No serious sequelae were observed. Some of the perforations were pan tympanic and included loss of nearly all of the tympanic membrane. They concluded that bFGF may be a simple, cost-effective treatment option for subjects with chronic tympanic membrane perforations. Human FGF-2 has been the most studied FGF isoform and its production and clinical applications in wound healing are well described.

Zhang and Lou (2012) present findings on the direct application of b-FGF on TMPs due to direct traumatic penetration. Of the 104 subjects, 51 were assigned to control (spontaneous healing) and 53 were assigned to FGF treatment. Statistically significant findings were observed. After 3 months, of the 93 patients analyzed, there was 100% closure of all TMPs in the experimental group compared to 77% in the control/spontaneous healing group. The treatment

group also had significantly faster closing times compared to the control group, on average 12.6 $\pm$ 1.2 days compared to 43.1 $\pm$ 2.5 days, respectively.

Lou, Wang & Yu (2014) present some of the most current research on the impact of basic fibroblast growth factor on human tympanic membrane perforations. In their 126-subject study, patients were dosed with either (Group 1) 2-3 drops of bFGF solution daily, approximately 0.1-0.15 mL or (Group 2) 5-6 drops of bFGF solution daily, approximately 0.25-0.3 mL. Follow up occurred at 3 months, at which time closure rate, closure time, and rate of otorrhea between the higher- and lower-dose groups were compared. This study found that, for perforations of the same size, subjects who received the higher dose showed significantly improved purulent otorrhea rate compared with the lower-dose group ($p < 0.01$). Closure rate of the middle-sized perforations did not differ significantly between the two groups ($p > 0.05$). The lower-dose group had a significantly shorter closure time (5 days) compared with the higher-dose group ($p < 0.05$). This study suggests that continued daily application of a lower dose of bFGF has the capacity to 1) shorten the closure time of human traumatic TMP and 2) avoids secondary purulent otorrhea.

While there is now more human data available that allows us to be confident about safety and efficacy of the clinical use of FGF-2, these are no studies conducted outside of the civilian population. In addition to conducting novel research in the United States, this study serves as a unique opportunity to advance current knowledge within the military population.

4. Study Objective

The primary outcome will be to determine the TMP closure efficacy of up to 3 treatments when compared to placebo.

4.1 Primary Objective

- To evaluate the efficacy of FGF-2 to repair chronic tympanic membrane perforations to complete closure as assessed by pre- and post-treatment photography when compared to placebo treated controls.

4.2 Secondary Objectives

- Determine the time to closure of tympanic membrane perforation for FGF-2 group and placebo group.
- Measure changes in pure-tone and speech discrimination scores in all study subjects.

- Determine mobility of tympanic membrane as measured by tympanometry in all study subjects.
- Observe salvage rate of FGF-2 treatment in subjects who failed placebo treatment.

5. Investigational Plan

5. 1 Overall Study Design and Plan Description

This study is a double blinded, placebo controlled phase II study evaluating the efficacy of FGF-2 for the treatment of chronic non-healing tympanic membrane perforations (TMP). The documentation of TM closure will be the main efficacy outcome measure. Pretreatment photographs will document the area of the TM perforation and allow measurement of surface areas of the TMP for comparison of pre- and post-treatment. The study will be divided into two phases, the Randomized Treatment phase (part A), and the Unblinded Crossover phase (part B). In part A of the study, subjects will be randomized 3:1 to receive FGF-2 or placebo treatment up to 3 treatments. Subjects that fail three study treatments will move on to part B of the study. Subjects who received placebo in part A and failed three placebo treatments will crossover to receive unblinded FGF-2 for up to 3 treatments. Subjects who received FGF-2 in part A and failed three experimental treatments will not have additional FGF-2 treatment, and will move on to study follow up.

The primary outcome for the study will be to determine the TMP closure efficacy of FGF-2 and placebo, with up to 3 treatments each. The OBD was determined to be 100μg of FGF-2 per 1 mL with an estimated dose of about 20 μg in 0.2ml in prior studies by Dr. Kanemaru (Kanemaru et al., 2011).

Secondary outcomes for the study include: (1) Determination of the time to closure of tympanic membrane perforation, (2) Measurement of changes in pure-tone and speech discrimination scores, (3) Determination mobility of tympanic membrane as measured by tympanometry, and (4) Observation of salvage rate of FGF-2 treatment in subjects who failed placebo treatment.

If both ears are affected by chronic perforation, only one ear will be initially enrolled in the study and receive treatment. The ear with the more significant symptoms will be the one enrolled in the study (i.e., greater conductive hearing loss). The non-study ear will not be enrolled or undergo additional treatments during the study period.

1) In an outpatient examination, the subject will be confirmed to have a tympanic membrane perforation by microscopic otoscopy and that no active infection and/or inflammation is present. Then a cotton ball soaked in 4% lidocaine jelly will be inserted with micro alligator forceps directly to the surface of the tympanic membrane under microscopic visualization.

2) After 15 minutes, the lidocaine infused cotton ball will be removed and the edge of the perforation roughened with a micropick to remove the epithelium circumferentially around the perforation edge.

3) A human fibroblast growth factor-2 (FGF-2) soaked pledget (approximately 20 μg in 0.2ml) of bioabsorbable gelatin sponge slightly larger than the tympanic membrane will then be placed against the lateral surface of the tympanic membrane to completely covered it. For the placebo group, only sterile water will be delivered by the pharmacy for saturation of the gelatin sponge.

4) The pledget will be secured in place with several drops of fibrin glue (Tisseel, McKesson Health Solutions, Auburndale, MA).

5) After the effects of the topical anesthetic have worn off, if any, the patient will be released with the following instructions:

a) Avoid violently sniffling or blowing the nose

b) Sneeze and cough naturally through the mouth without occluding the mouth with the hand.

c) Use cotton balls covered with petroleum jelly on the outer surface when washing the hair and bathing, to keep water out of the ear.

d) Avoid using a hearing aid in the treated ear.

e) Avoid flying, using elevators in tall buildings, and other situations involving substantial changes in air pressure.

f) Avoid swimming.

6) The subject will then come back at three weeks (+/- 7 days) for a follow- up appointment. The tympanic membrane will be examined to see if the perforation has closed and photographed in the same fashion as during the IMP or initial administration visit. Additional otologic tests will be performed during this follow up exam as well (See 13.1 Schedule of Assessments). If the tympanic

membrane has closed, then the participant will be seen again in approximately 2 months (+/-7 days) for a final study visit.

However, if the perforation has not closed, then the FGF-2 or placebo application will be duplicated in the same fashion at this follow up visit. The subject will then be seen again at three weeks (+/- 7 days). The tympanic membrane will be assessed again. If the TMP is not healed, then a third application of the FGF-2 or placebo will be applied again at this visit. Additional otologic tests will be performed once again. If the TMP heals, then the subject will be seen again following this visit, or at approximately two months (+/-7 days).

Once the subject completes Visit 1, Visit 2, and Visit 3, the subject will come back for Visit 4 (+/- 7 days). During this visit, the perforation will be examined again. If the perforation has not closed completely at this visit, then the blind on the subject's randomization status will be broken. If the subject was randomized to placebo, s/he will be crossed over to receive unblinded FGG-2 treatment. If the subject was randomized to FGF-2 treatment, s/he will receive no further study treatment and return in two months (+/- 7 days) for the final follow up visit.

If crossed-over to unblinded FGF-2 treatment, the subject may be given up to three additional treatments. The follow up visits will occur three weeks (+/- 7 days) and the same otologic procedures will be conducted as mentioned before (See Section 13.1, Schedule of Assessments). If the perforation is found to be closed during these visits or when the subject has completed the maximum number of study visits without perforation closure, then the subject will report in 2 months (+/- 7 days) for a final visit. The study will be completed for reporting purposes after all subjects have completed the 2 month (+/- 7 days) follow up final visit.

6. Discussion of Study Design

The investigator and the subject will be blinded to treatment assignment during the Randomized Treatment phase. Subjects whose perforations fail to close during the randomized treatment phase will crossover to the Unblinded Crossover Phase. During the crossover phase, only those subjects who failed placebo will receive unblinded FGF-2 treatment. The rationale for using a dose of 20 μg in 0.2ml is based on the maximum effective dosages as reported in humans of TMR closure with FGF-2 (Kanemaru et al., 2011).

The subjects, physician, and other study staff members will remain blinded to the treatment arm during the randomized treatment phase as the pharmacy will oversee the randomization process. During the Randomized Treatment phase, the pharmacy will provide the appropriately blinded vial of either FGF-2 or placebo solution as needed upon the request from the physician. Only those subjects who fail the randomized treatment phase will crossover into the unblinded crossover phase. Documentation of the cross-over will be made in the Case Report Forms as well as in the randomization log managed centrally by the dispensing pharmacy.

7. Selection of Study Population

7.1 Inclusion Criteria

The subjects must meet the following inclusion criteria:

1. Age 18 years and older
2. Dry tympanic membrane perforation of greater than 3 months duration
3. If female, post-menopausal or sterile, or if she is of child-bearing potential, must have a negative beta-HCG test and must be using an adequate form of birth control such as birth control pills, birth control implants, intrauterine devices (IUDs), diaphragms, or condoms throughout the clinical trial.

7.2 Exclusion Criteria

The presence of any of the following excludes a subject from study enrollment:

1. Active otitis media or chronic otorrhea from the middle ear
2. Subjects receiving radiation therapy, corticosteroids, immunosuppressive agents or chemotherapy
3. Subjects who, at study entry, are taking systemic antibiotics
4. Subjects who are immunosuppressed
5. Subjects experiencing bacterial or viral infection or who may otherwise be febrile
6. Active alcohol or drug abuse within 6 months prior to study entry
7. Significant medical condition that could prevent full participation in the procedures required for the study

8. Not able to complete all study procedures
9. Subjects with known allergies to porcine collagen
10. Subjects who have cholesteatoma mass in the tympanic cavity
11. Subjects whose total perforation cannot be seen by an endoscope
12. Subjects with inadequately controlled diabetes mellitus (HbA1c 6.9% or higher). HbA1c will be measured at baseline to assure entry criteria are met.
13. Subjects with a history of malignant ear canal tumors within 3 years of screening for eligibility
14. Subjects who are judged by the investigator or sub-investigator(s) to be inappropriate to participate in the study

7.3 Removal of Subjects from Treatment or Assessment

Reasons for permanent discontinuation include the following:

If three treatments do not result in complete closure, subsequent treatments will not be administered if any ear complications occur in the treated ear, including but not limited to, infection, requires other surgery, moderate to severe pain, moderate to severe irritation, clinically significant hearing loss. All subjects will complete all safety follow-up visits.

7.4 Premature Termination

Potential reasons for removal of subjects from treatment include the following:

- Withdrawal of consent
- Adverse Events
- Patient is lost to follow-up
- Termination of study by the Sponsor
- Pregnancy

8. Treatments

8.1 Identity of Investigational Product(s)

FGF-2 is manufactured by Kaken Pharmaceutical Co., Ltd. and will be supplied by Nobelpharma Co Ltd.

The FGF-2 method of use comprises a pledget of gelatin sponge, with human fibroblast growth factor-2 (FGF-2) added to the pledget just before topical application to the tympanic membrane, and the use of fibrin glue used for applications in ear surgery. The concentration of the FGF-2 solution will be 100μg of FGF-2 per 1 mL with an estimated dose of about 20 μg in 0.2ml.

9. Handling and Disposition of Study Drug

9.1 Packaging and Labeling

All packaging and labeling operations will be performed according to the requirements of Directive 2001/20/EC[1] and in accordance with Good Manufacturing Practice for Medicinal Products.

9. Conditions for Storage and Use

FGF-2 is to be stored in a cool location. The expiration date is as indicated on outer box and label. After reconstituting, FGF-2 is to be kept in a cool, dark place at ≤10°C and used within weeks. In this clinical study, the product will be prepared as needed, with 1 vial used for each case in this clinical study.

10.1 Instructions to the Pharmacist

Lyophilized FGF-2 in the amount of 250 μg will be supplied in vials along with 2.5 ml of sterile water to the clinical site pharmacy for preparing dosing levels at 10 μg/mL. The drug kits will also provide dilution instructions to the pharmacist. The control solution will consist of the sterile water without any FGF-2.

Fibrinogen tissue glue will be supplied in 4 vials to the pharmacist. The first powdered fibrinogen (vial 1) is reconstituted with all of the aprotinin solution (vial 2) to prepare Solution A. The thrombin powder (vial 3) is reconstituted with a volume of calcium chloride solution (vial 4) equal to the volume of the aprotinin solution to prepare solution B. Several drops of solution A and then solution B are applied in layers to the lateral surface of the gelatin sponge in the medial external auditory canal at the site of the adhesion.

10.2 Method of Assigning Subjects to Treatment – Randomization

To generate the randomization scheme, a random number generator program known as Research Randomizer will be used (Urbaniak & Plous, 2013). Subjects will be randomly

assigned to unequal treatment groups in a 3:1 ratio. A random number scheme will be created containing 15 sets of numbers with 4 unique numbers per set (1-4) [total of 75 numbers corresponding to 75 subjects]. Of the 4 numbers per set, patients assigned to numbers 1, 2, or 3 will receive FGF-2 and patients assigned to number 4 will receive placebo. Once the random numbers are generated, the pharmacy will create an Excel spreadsheet with the FGF-2 and placebo assignments corresponding to the numbers that were generated.

Randomization assignments will be done centrally by the MEE pharmacy. Subjects are assigned to treatment by placing the subject's name on the next available line of the spreadsheet. For subjects treated at MEE, the pharmacist uses this information to prepare the proper treatment for the subject. For subjects at the Willford Hall location, the randomization assignment will be communicated to designated staff at Willford Hall via secure email.

10.3 Selection and Timing of Dose for Each Patient

Dosing will be initiated after the informed consent has been obtained and all necessary study eligibility assessments have been completed. Dosing will be according to the schedule of visits.

11.1 Blinding

This is a double blinded, placebo controlled study.

10. Prior and Concomitant Therapy

All prior and concomitant therapies will be assessed by the principal investigator if needed.

12.1 Prohibited Medications or Therapies During Study

Medications having the potential to interfere with the evaluation of efficacy are excluded throughout the trial and include:

- Antibiotic ear drops
- Steroid ear drops

12.2 Treatment Compliance

Treatment compliance will be assessed by the principal investigator following administration of FGF-2.

13.1 Schedule of Assessments

Visit	0	1	2	3	4	5	6	7	Final Visit
	Screening	Day 1	Day 22 (± 7 days)	Day 43 (± 7 days) As Needed	Day 64 [5] (± 7 days) As Needed	Day 85 [6] (± 7 days) As Needed	Day 106 [6] (± 7 days) As Needed	Day 127 [6] (± 7 days) As Needed	F/U [7] (±7 days)
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Medical History Related to the Tympanic Membrane	X								
Otologic Exam	X								X
Vital Signs	X[2]	X[2]	X	X	X	X	X	X	X
Laboratory Evaluation *[CBC, Basic Metabolic Panel, HbA1C]*	X								X
Pregnancy Test[3]	X								
Prior/Concomitant Medication	X	X	X	X	X	X	X	X	X
TM photography	X[1,2]	X[1,2]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X
Audiology		X[1, 2]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X
Tympanometry									X
Study Drug Administration		X	X[4]	X[4]	X[4]	X[4]	X[4]		
Review of Adverse Events		X	X	X	X	X	X	X	X

[1]To be completed prior to administration of the investigational drug.
[2] This may be performed during the screening visit or during Day 1 Visit. Note: The first audiogram may have been performed within 60 days prior to subjects' enrollment.
[3] Women of child bearing potential will be required to have a negative pregnancy test. Either serum or urine tests are acceptable. Until study completion, adequate form of contraception must be ensured.
[4] Treatment given if the perforation has not closed at this visit.
[5] If the perforation has not closed at this visit, then the subject will be assigned to the other treatment group. The subject will then follow the same schedule of procedures as stated for Visit 5, Visit 6, Visit 7, and Final Visit.
[6] These visits will be completed by subjects that have been assigned to the other treatment arm and whose perforation still has not closed.
[7]The final study visit occurs 2 months (+/- 7 days) after the subject's last follow-up visit. The subject's last follow-up visit occurs when the subject's perforation is determined to be closed, or when the subject has completed the maximum number of study visits without perforation closure

13.2 Efficacy Measurements

- Dose limiting toxicity/Optimal Biologic Dose is as defined in section 7.1
- TM closure is defined as complete closure of the tympanic membrane durable at both 21-days (±7 days) and the test-of-cure Visit 5.
- TM Photographs will be obtained at every visit. A series containing pre- and post-treatment images will be digitally secured with Leica M320 high definition digital camera mounted with the Leica Microscope or with a 2.7 mm rigid Storz endoscope.
- Audiometric studies including pure tones (0.25, 0.5, 1, 2, 4, and 8 kHz) and speech discrimination scores will be recorded pretreatment and post treatment. A change of >10 dB across two frequencies or a change in speech discrimination of > 20% will be considered clinically significant on an NU-50 word list.
- Mobility of the tympanic membrane as measured by a Grayson Stadler GSI 37 Autotymp (Madison, WI) or equivalent tympanometer will be recorded when the TM appears healed otoscopically.

13.3 Safety Measurements

- Adverse events by severity and causality as described in section 7.7
- Laboratory evaluations including SMA-21 and CBC with differentials.
- Clinically meaningful changes in vital signs including temperature, blood pressure, weight, pulse, and respiratory rate

13.4 Pharmacokinetic and Bioanalytical Methods

No pharmacokinetics will be performed.

13.5 Treatment Requirements

Subjects must complete at least 1 treatment and have a follow up visit at 21 days (± 7 days) to be evaluated for efficacy.

13. 6 Timing of the Therapeutic Dose

Therapy will be administered every 21 days (± 7 days) for up to three applications or until there is complete closure of the perforation. A cross over will occur if the perforation is

not completely closed at Visit 4. A Final Visit will occur in 2 months (+ 7 days) following the subject's last appointment.

14. Study Conduct

14. 1 Visit 0, Screening

The following procedures will be performed:

- Administration of Informed Consent Form
- Review of inclusion and exclusion criteria
- Medical history of the Tympanic Membrane
- Otologic Exam
- Vital Signs
- Laboratory evaluation (hematology and chemistry): *CBC, Basic Metabolic Panel Test, HbA1c*
- Pregnancy Test if applicable
- Review of Prior/ Concomitant medication(s)
- Optional: Study drug administration (if all eligibility requirements can be confirmed at this visit)
- Optional: TM photography
- Optional: Audiogram

14. 2 Visit 1, Day 1, IMP Administration

The following procedures will be performed:

- Review of inclusion and exclusion criteria
- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography- *if not done at the time of screening*
- Audiogram- *if not done at the time of screening*
- Study Drug Administration- *if not done at the time of screening*
- Assessment of Possible Adverse events

14.3 Visit 2, Day 22 (± 7 Days)

The following procedures will be performed:

- Study drug administration - *if the TMP is not completely closed*
- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Assessment of Possible Adverse events

14.3 Visit 3, Day 43 (± 7 Days)

The following procedures will be performed:

- Study drug administration- *if the TMP is not completely closed*

- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Assessment of Possible Adverse events

14.4 Visit 4, 64 days after the first treatment (±7 Days)

The following procedures will be performed:

- Study Drug administration- *If the TMP is not completely closed, then the blind on the subject's randomization status will be broken. Placebo subjects will be crossed over to FGF-2 treatment arm at this visit.*
- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Assessment of Possible Adverse events

14. 5 Visit 5, 85 days after first treatment (±7 Days)

This is a follow up visit for all subjects who were crossed over to the other treatment group at Visit 4. The following procedures will be performed:

- Study Drug administration- *if the TMP is not completely closed*
- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Assessment of Possible Adverse events

14. 6 Visit 6, 106 days after first treatment (±7 Days)

This is a follow up visit for all subjects who were crossed over to the other treatment group at Visit 4. The following procedures will be performed:

- Study Drug administration- *if the TMP is not completely closed*
- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Assessment of Possible Adverse events

14. 7 Visit 7, 127 days after first treatment (±7 Days)

This is a follow up visit for all subjects who were crossed over to the other treatment group at Visit 4. No treatment medication will be given. The following procedures will be performed:

- Vital Signs
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram

- Assessment of Possible Adverse events

14. 8 Final Visit (2 Months Following Last Appointment, ± 7 Days)

In order to complete the study, a final follow up visit will be completed. The following procedures will be performed:

- Otolgic Exam
- Vital Signs
- Laboratory evaluation (hematology and chemistry): *CBC, Basic Metabolic Panel Test, HbA1c*
- Review of Prior/ Concomitant medication(s)
- TM photography
- Audiogram
- Tympanometry
- Assessment of Possible Adverse events

15. Primary Safety and Efficacy Variable(s)

15.1 Adverse Events (AE)

An Adverse Event will be considered any unfavorable or unintended change in structure, function, signs, or symptoms temporally associated with the use of a medicinal product experienced by a person administered a pharmaceutical product, whether or not a causal relationship with the product has been established. Clinically significant laboratory abnormalities may be considered AEs if deemed appropriate by the Investigator. Worsening of a pre-existing condition is also considered an AE as is the discovery of an abnormal finding during physical exam that was not included in the medical history.

Subjects will be encouraged to spontaneously report any AE. Study personnel will ask open-ended questions to obtain information about AEs at every visit. Date and time of onset and resolution (if applicable) of the AE will be documented.

15.2 Causality

Events will be considered drug related if classified by the Investigator as possible, probable, or definite. Association of events to the study drug will be made using the following definitions:

Term	Definition
Definitely Not	The event is definitely not associated with study medication.
Probably Not	The temporal association, patient history, or clinical condition is such that the study medication is not likely to have had an association with the observed event.
Possible	The event: a) follows a reasonable temporal association with the study medication administration, but b) could have been produced by the patient's clinical condition or other therapy.
Probable	The event: a) follows a reasonable temporal association with the study medication, b) abates upon discontinuation of study medication, and c) can't be reasonably explained by the patient's clinical condition or other therapy.
Definite	The event: a) follows a reasonable temporal association with the study medication, b) abates upon discontinuation of study medication, c) cannot be reasonably explained by the patient's clinical condition or other therapy, and d) reappears on re-exposure to the study medication.
Unknown	Not enough information exists for the assessment of causality at the time of occurrence.

15.3 Severity

Signs and symptoms will be graded by the Investigator using the NCI Common Toxicity Criteria and graded as mild, moderate, severe, or life-threatening according to the following definitions:

Grade	Definition
Mild	Causing no limitation of usual activity

Grade	Definition
Moderate	Causing some limitations of usual activities
Severe	Causing inability to carry out usual activities
Life-Threatening	Subject was at immediate risk of death from the event

15.4 Serious Adverse Event (SAE)

A Serious Adverse Event is an AE that:

- Is fatal
- Is life-threatening, meaning the subject was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more serious form or progressed, might have caused death
- Is a persistent or significant disability or incapacity
- Requires or prolongs inpatient hospitalization. [Inpatient hospitalization will be considered a hospitalization that is longer than 24 hours, or a hospitalization that requires an intervention to treat emergent symptomatology (non-diagnostic)]
- Is a congenital anomaly or birth defect.

Other important medical events may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes as listed (# 1-5 above) in this definition.

All SAEs, including death, due to any cause that occurs during this study and until 30 days after the last dose of study medication, whether or not expected and regardless of causality, must be reported to the Medical Monitor immediately upon discovery of the event, using an SAE Form, by fax and, if necessary, by phone.

The Medical Monitor will advise the Investigator regarding the nature of any further information or documentation that is required. The Investigator should provide the following documentation at the time of notification if available:

- SAE Form
- AE (CRF) page
- Concomitant and support medication pages
- Relevant diagnostic reports
- Relevant laboratory reports
- Admission notes
- Hospital discharge summary (when available).

Nobelpharma will make available to the Investigator any reports of serious, unexpected, and drug-related AEs by fax within eight (8) days of notification of the event using a *MedWatch 3500A Form*. The Investigator must promptly (within 2 days) inform the IRB of such events and retain a copy of the notification in the site's Regulatory Binder.

All SAEs must be followed until the event resolves, returns to baseline or, in the opinion of the Investigator, becomes stable. Hospitalization of subjects for the administration of chemotherapy will not be considered an SAE.

16. Data Quality Assurance

16. 1 Source Data and Records

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, data sheets provided by the sponsor or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IRB review(s) and regulatory inspection(s), with direct access to all the required source records.

In the event the Investigator retires, relocates or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to any other person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor. The Investigator must contact the Sponsor prior to disposal of any records related to this study.

16. 2 Reporting of Results

The Case Report Form (CRF) is an integral part of the study and subsequent reports. The CRF must be used to capture all study data recorded in the subject's medical record. The CRF must be kept current to reflect subject status during the course of the study. Only a Subject screening and randomization number and subject initials will be used to identify the Subject.

The principal investigator and/or study coordinator is responsible for performing on-site monitoring at regular intervals throughout the study to verify adherence to the protocol; verify adherence to local regulations on the conduct of clinical research; and ensure completeness, accuracy, and consistency of the data entered in the CRF.

The Data Safety and Monitoring Committee and the principal investigator will monitor completed Case Report Forms (CRFs). A case report form will be provided for each screened subject.

All protocol-required information collected during the study must be entered by the Investigator, or designated representative, in the CRF. All details of the CRF completion and correction will be explained to the investigator.

If the Investigator authorizes other persons to make entries in the CRF, the names, positions, and signatures of these persons must be supplied to the sponsor.

The Investigator, or designated representative, should complete the CRF as soon as possible after information is collected, preferably on the same day that a study Subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. By design, an explanation must be provided for all missing data, altered data, and/or out of range data.

The completed case report form must be reviewed and signed by the Investigator named in the study protocol or by a designated sub investigator.

Final monitored and audited CRFs will be provided to the sites at the end of the study in the format of a PDF file.

16. 3 Confidentiality of Subject Data

The investigator will ensure that the confidentiality of the subjects' data will be preserved. In the CRF or any other documents submitted to the sponsor, the subjects will not be identified by their names, but by an identification system, which consists of their initials and number in the study. The investigator will maintain documents not meant for submission to the sponsor, e.g., the confidential subject identification code and the signed informed consent forms, in strict confidence.

17. Statistical Methods and Determination of Sample Size

17. 1 Determination of Sample Size

The target enrollment of 60 subjects will provide sufficient power while also accounting for potential retention issues. With recruitment of only 44 subjects, we will reach a power of .80. This gives room for some attrition, although we think it will not be more than 5%.

17. 2 Subject Populations

The following populations will be used for the analysis of data for this study:

Per Protocol (PP) Population: All subjects who have been selected and received the specified drug at Visit 1 and do not have any important protocol deviations will be included in the PP population. The PP population will be the primary population for the analysis of efficacy.

Safety Population: All subjects who received the drug will be included in the safety population. The safety population will be the primary population for analysis of demographic, accountability, and safety measures.

17. 3 Subject Disposition

The number and percentage of subjects in each of the analysis populations and number and percentage of subjects who discontinued the study prematurely will be summarized by treatment and overall. The reasons for early study termination will also be presented. Details of study disposition and inclusion/exclusion criteria for all subjects will be listed.

17. 4 Demographics and Baseline Characteristics

Demographic and baseline characteristics including age, gender, race and relevant baseline medical conditions will be summarized by treatment and overall. Descriptive statistics will be presented, but no statistical testing will be performed. Subject demographic and baseline characteristics and medical conditions will be listed.

17. 5 Efficacy Analysis

Efficacy data from the Randomized Treatment phase we be evaluated by inspection, using student T-test, Cochran Mantel Hansel, or Kruskal-Wallis tests as appropriate; without conservation of alpha at 0.05. An interim analysis will be completed after approximately 50% of the study subjects (n=30) have been accrued.

17.6 Secondary Efficacy Analysis

Secondary analysis of categorical data will be performed using Fisher's exact test for paired samples.

18. Safety Analysis

Safety will be assessed by evaluation of adverse events and clinical laboratory results and derived as data sets and frequency methods.

An independent Data and Safety Monitoring Board (DSMB) will follow the DSMB charter for this study. The DSMB Charter is commensurate with the level of risk of the study to its participants, complexity of the protocol, and ensure compliance with the principles and process of informed consent as well as privacy related regulations (e.g., HIPAA); and to monitor safety by recording (un-)expected adverse events (AEs) and their severity and attribution. The DSMB Charter includes a plan for periodic reviews of primary results and summary data as well as assessment of completeness and quality of collected data. All unexpected and/or serious adverse events as well as premature study withdrawals will be brought to the attention of the DSMB as early as possible in order for the DSMB to make judgments about participant safety, study modifications, and/or continuation. Not beyond the commencement of the proposed subsequent dose escalated cohorts, the DSMB will review study status to determine study progression. These will be based upon discussions between the research team and DSMB, and the decision of the board; no set stopping or escalating rules will be employed. The DSMB membership list is as follows:

Edwin Choy, MD, PhD - Chair and Research Monitor
MGH, Dana Farber/Harvard Cancer Center
Division of Hematology Oncology
55 Fruit Street
Boston MA 02114
Phone: (617) 643-0230
Fax: (617) 724-3166
Email: echoy@partners.org

Christopher Moertel, MD, - Member
University of Minnesota
Department of Pediatrics
MMC 484
420 Delaware Street S.E.
Minneapolis, MN 55455
Phone: (612) 625-3229
Fax: (612) 626-2815
Email: moert001@umn.edu

Domenic Reda, PhD, Member (Statistician)
Director, Cooperative Studies Program Coordinating Center
Hines VA Hospital, CSPCC (151K)
5000 South 5th Avenue, Building 1, Room B240,
Hines, IL 60141-3030
Phone: 708-202-5853
Fax: 708-202-2116
domenic.reda@va.gov

19. Research Monitor

The Research Monitor, Edwin Choy MD, PhD, is responsible to oversee the safety of the research and report observations/findings to the Human Studies Committee (HSC) or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the HSC. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the HSC can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the HSC or other designated official and the HRPO.

20. Adverse Events

Adverse events will be coded to system organ class and preferred term using MedDRA**.** All adverse events occurring after the initiation of the study treatment (treatment emergent adverse events) will be reported, including events present at baseline that worsened during the study.

Adverse events will be summarized by treatment group to provide visual comparison among the treatment groups with respect to incidence of adverse events (the number of subjects reporting at least one episode of a specific adverse event), incidence of adverse events by severity within body system, incidence of adverse events by attribution within body system, and incidence of adverse events causing withdrawal and incidence of serious adverse events. Regarding severity and attribution summaries, the most extreme outcome (highest severity and closest to study drug related) will be used for those subjects who experience the same adverse event on more than one occasion.

Written narratives will be provided for all serious, unexpected or other significant adverse events that are judged to be of special interest because of their clinical importance.

21. Clinical Laboratory

Clinical laboratory results will be summarized with descriptive statistics at baseline each study timepoint and with shifts from baseline.

22. Data Handling and Recordkeeping

22.1 Case Report Form (CRF)

MEEI will be the central location for data processing and management utilizing the biostatistician on site. All protocol-required information collected during the study will be entered by the investigator, or designated representative, in the appropriate study forms and patient medical records. The investigator, or designated representative, should complete the forms as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedures. Any outstanding entries will be completed immediately after the final examination. By design, an explanation must be provided for all missing data, altered data, and/or out of range data. The completed case report form will be reviewed and signed by the investigator named in the study protocol or by a designated sub- investigator.

A Case Report Form will be completed for each subject for each study visit. Subjects will not be identified by name on any study documents. Any requested information that is not obtained as specified in the protocol should have an explanation for the omission noted on the applicable study form.

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, data sheets provided by the sponsor or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IRB review(s) and regulatory inspection(s), with direct access to all the required source records. Hard copies of any individual's study documents will be kept in separate subject binders in the locked office of the study coordinator at each site.

22.2 Patient Registry

No patient registry will be maintained.

23. Ethics

23.1 Institutional Review Board (IRB) / Ethics Committee

Before the start of the study, the study Protocol, Informed Consent Form (ICF), and/or other appropriate documents will be submitted to the IRB in accordance with local legal requirements. It is the responsibility of the Investigator to ensure that all aspects of the IRB review are conducted in accordance with current local and U.S. regulations. Phage and the Investigator must inform each other in writing that all ethical and legal requirements have been met before the first patient is enrolled at the study site.

24. Ethical Conduct of the Study

The study will be conducted in accordance with the ethical principles of the current version of the Declaration of Helsinki. Sponsor and Investigator will comply with their responsibilities as defined in 21 CFR 312.50-312.70 [Appendix D] and ICH Guidance for Industry: E6 Good Clinical Practice (April 1996).

24.1 Subject Information and Consent

The Investigator or delegated research staff will obtain informed consent from each subject enrolled in the study, in accordance with the U.S. Food and Drug Administration (FDA) regulations 21 CFR 50.20 - 50.27 and the laws and regulations of the state in which the investigation is being conducted [Appendix C].

Informed consent will be obtained prior to the initiation of any study related procedures including screening. Screening procedures for this study include vital signs, laboratory evaluations, a pregnancy test (if applicable), and a photograph of the tympanic membrane. Non- study related procedures that will be part of the routine medical care include a comprehensive medical history of the subject's tympanic membrane, otologic exams, and audiograms. Audiograms that have been completed within 60 days prior to enrollment may be reviewed by the subject's study physician. Should a Protocol amendment be made, the subject ICF may be revised to reflect the changes of the Protocol. If the ICF is revised, it is the responsibility of the Investigator to ensure that an amended ICF is reviewed and approved by the IRB and signed by all subjects subsequently entered in the study.

This study will be consenting subjects that are non-English speaking. In these cases, the person obtaining the consent will present the IRB-approval English version of the consent form orally to the subject through a medical interpreter fluent in English and in the language that is understandable to the subject. If an Interpreter is not available, then video interpreting will be used. The subject will be given a HSC-approved 'short form' consent document in the language understandable to him/her to read.The entire consent process will be witnessed ideally by an individual who is fluent in both English and the language understandable to the subject. The witness who is not bilingual will observe that the presentation of the information in the consent form is in the language understandable to the subject and will verify the subject has had the opportunity to ask and receive answers to questions in a non-coercive, private, and unhurried setting. The IRB-approved English version of the consent form will be signed by the person obtaining informed consent and the witness to the consent process. The written translation of the *'*short form*'* will be signed by the subject. If the witness is bilingual, then the witness can also sign the short form. The subject will be given signed copies of both the IRB-approved English version of the consent form and the written translation of the 'short form' consent document. The original signed English version of the consent form with the original signed written translation of the '*short form'* document will then be placed in the subject's

research record.

24.2 Protocol Amendments

Amendments will be made only in exceptional cases once the study has started at the discretion of the Investigator and with confirmation from Nobelpharma Co Ltd. Changes must be agreed to in writing, and signed by all parties concerned. The changes then become part of the study Protocol. Any changes in the Protocol, even local/site requirements, will be written in an amendment. The IRB must be informed of amendments and, if necessary, approval must be sought for ethical aspects. Approval of amendments must also be obtained from the local regulatory authority, if necessary.

25. Confidentiality

All local legal requirements regarding data protection will be enforced. All study findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from Phage. The anonymity of participating subjects must be maintained. Throughout documentation and evaluation, the subjects will be identified on CRFs and other documents submitted to Phage by their initials, birth date, and their subject number. Documents that are not to be submitted to Phage, and which identify the subject (i.e. the signed ICF), must be maintained in confidence by the Investigator onsite. The subjects will be told that all study findings will be stored and handled in strictest confidence, according to local requirements. Subjects will be informed that authorized research investigators and agents of the FDA, other recognized regulatory authorities, and authorized representatives of the Sponsor, Phage, have the right to inspect their medical records.

26. Investigators and Study Administrative Structure

This is a Phase 2 trial involving two sites located in the United States. The Investigator(s) responsible for the conduct of the study at their site, are responsible for maintaining all local IRB approvals and documenting all required information per regulations of guidelines stipulated by the respective Institutional Review Boards and the DoD. Participants will include trained otologists in the MEE clinic and at Willford Hall AFB (Dr. Carols Esquivel)

27. Sponsor Contacts

All questions regarding the enrollment of subjects, regulatory requirements for the conduct of the study, safety reporting, or study conduct should be addressed to the Medical Monitor:

28. Maintenance of Study Records

The Investigator must retain a copy of all study documents, including reports to the IRB, regulatory authorities, and Phage in accordance with FDA regulations.

The Investigator must maintain study documents:

- For a minimum of two (2) years following the date the marketing application (NDA/BLA) is approved for the indication for which the drug was investigated; or,
- For a minimum of two (2) years following the release date of the final report, if no marketing application is to be filed by Phage, or if the marketing application is not approved for the indication for which the drug was investigated or is discontinued and the FDA has been notified; or,
- For a minimum of fifteen (15) years after the completion or discontinuation of the study to be filed in support of the registration in the European Union.

If the Investigator relocates, retires for any reason, or withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to Phage. The Investigator must obtain Phage's written permission before disposing of or transferring any records.

29. Final Report

Upon terminating the study, the Investigator will submit a final report to the IRB in keeping with local IRB regulations. This report should include any deviations from the Protocol, the number and types of subjects evaluated, the number of subjects who discontinued, including reasons, results of the study, AEs, and a conclusion summarizing the results.

If requested by the Investigator, at the completion of the study and following analysis of the data, Phage will supply a tabulated listing of data and a final clinical statistical report. A

copy of the final study report and corrected CRFs, including a receipt to be returned to the Phage Clinical Monitor, will be provided to each Investigator following its release by Phage.

30. Publication and Use of Study Findings

By signing the study Protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement. Phage will prepare an *Integrated Clinical/Statistical Report*. Any publication/presentation of data must include the entire study population. Submission of data for publication/presentation will be coordinated and approved by Phage in collaboration with the Investigator. Phage will determine authorship of any publication by enrollment in consultation with the Principal Investigator.

31. Liability and Insurance

NobelPharma Co. will take out reasonable third-party liability insurance coverage in accordance with all local indemnification requirements. The civil liability of the Investigator, the persons instructed by him, the hospital, practice, or institute in which they are employed, and the liability of the Sponsor in respect of financial loss due to personal injury and other damage, which may arise as a result of the carrying out of this study, are governed by the applicable law. As a precautionary measure, the Investigator, the persons instructed by him or her and the hospital, practice or institute are included in such cover in regards to work done by them in carrying out this study to the extent that the claims are not covered by their own professional indemnity insurance. The Sponsor will arrange for subjects participating in this study to be insured against financial loss due to personal injury caused by the pharmaceutical products being tested or by medical steps taken in the course of the study. The Sponsor in accordance with regulations in the country concerned takes out such insurance. To the extent that payments are made under such insurance, the right to claim damages from the Sponsor extinguishes.

32. References

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Appendix A: NCI Common Toxicity Criteria

Please reference the National Cancer Institute Cancer Therapy Evaluation Program's Common Toxicity Criteria Manual, Version 4.0.

Retrieved from,

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcmanual_v4_10-4-99.pdf

Appendix B: FDA Regulations

Please reference the electronic code of federal regulations for the most up-to-date federal regulations on the protection of human subjects:

21 CFR part 50

http://www.ecfr.gov/cgi-bin/text-idx?SID=b0c39f9f49cefa4e1423e8b274183684&mc=true&tpl=/ecfrbrowse/Title21/21cfr50_main_02.tpl

21 CFR part 56

http://www.ecfr.gov/cgi-bin/text-idx?SID=b0c39f9f49cefa4e1423e8b274183684&mc=true&node=pt21.1.56&rgn=div5