Chapter 5
Development of Drugs for Noise-Induced Hearing Loss

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Abstract Current efforts to translate laboratory findings into clinical drugs for preventing or treating sensorineural hearing loss (SNHL) are reviewed in this chapter. Since no drugs have been approved for any inner ear indication involving SNHL, including tinnitus, this is a novel area of both clinical development and regulatory oversight. An overview of FDA guidance, and sponsor interactions or meetings with the FDA, involving preclinical data to clinical trial design and regulatory approval are reviewed. Specific steps on how to evaluate the safety and efficacy of an investigational new drug through a series of well-controlled preclinical (nonhuman) and clinical (human) studies are provided. The information presented is designed to assist researchers and clinicians in mapping out and executing an effective drug development program. In this chapter, regulatory considerations for the development of a new or existing drug that reduces, mitigates, prevents, or treats noise-induced hearing loss (NIHL) are reviewed, including both preclinical and clinical FDA interactions, followed by a detailed discussion of the investigational drugs that are furthest along in clinical testing.

Keywords Clinical trial · FDA · Investigational new drug · Noise-induced hearing loss · Otoprotection

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5.1 Introduction

In developing drugs for preventing or alleviating sensorineural hearing loss (SNHL), an important question is whether the use of the drug will be ethical. Although seemingly obvious, this question bears significant regulatory meaning. In the US Food and Drug Administration (FDA), in Europe's European Medicines Agency (EMA), and in Japan's Pharmaceutical and Food Safety Bureau (PFSB), the concept of an "ethical drug" is largely agreed to be a drug that has proven to be both safe and efficacious. This is demonstrated through a series of well-controlled preclinical (nonhuman), and clinical (human) studies aimed at understanding the pharmacologic and toxicologic effects of the investigational drug for a specific disease, disorder, or condition. Although the regulatory environment varies from country to country, the international community has adopted guidelines set forth by the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use with regard to drug development and marketing.

In the United States, FDA guidance is clear. An investigational new drug (IND) application is required when clinical studies intended to evaluate the safety and efficacy of an unapproved drug are proposed. In addition, any clinical evaluation that changes the dose, route of administration, formulation, or indication generally requires an IND or a waiver from the FDA. Research groups seeking to develop ethical drugs for the prevention and treatment of SNHL are encouraged to review and understand the regulatory aspects governing their development program. One helpful resource to be consulted early in the process is the Center for Drug Evaluation and Research (CDER) at the FDA, which offers a Pre-Investigational New Drug Application (PIND) Consultation Program. For investigators at academic institutions, assistance with regulatory support or other relevant resources may be available through a clinical trial management office or clinical translational research center.

Before beginning the costly and lengthy process of performing the requisite preclinical safety studies in support of an IND, the investigational compound should be well vetted for safety and efficacy in at least one relevant animal model of the human condition or disease. Developing a new drug is expensive and it requires years of sustained effort. Current estimates for the costs of developing a new chemical entity (NCE), a new drug that has never been approved by the FDA, can exceed $1 billion. Higher costs are associated with biologics (e.g., recombinant proteins, gene therapy) and agents that act on the central nervous system (CNS) (DiMasi et al. 2003; DiMasi and Grabowski 2007), since clinical testing typically requires 10+ years (see Fig. 5.1; for additional discussion, see Morgan et al. 2011). Setting a foundation based on solid preclinical evidence is crucial. Publication of results and methods from animal studies affords a greater potential for useful discoveries to be validated by independent parties. Before publicly disclosing or otherwise sharing new discoveries, however, intellectual property protection must be considered. The importance of protecting data generated in

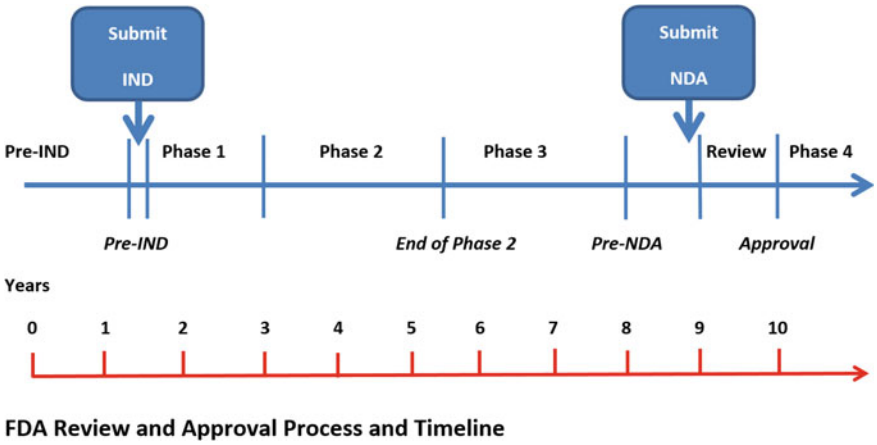

Fig. 5.1 The timeline for the FDA review and approval process. Preclinical research generating data required for the filing of an investigational new drug (IND) application are shown here as 18 months, which is likely an idealized situation. In many cases, preclinical research and the necessary toxicological, pharmacological, and pharmacodynamic safety research may proceed over multiple years before first-in-man studies. Phase 1 studies are typically limited to safety and Pk/PD parameters, whereas Phase 2 studies include both safety and efficacy. Phase 3 studies are the larger safety and efficacy registration studies that are necessary to seek permission for health claims from the US FDA

preclinical testing is not discussed in detail in this chapter but is an essential step in the translational process, and Chap. 2 by Le Prell should be reviewed for discussion on this step. In this chapter, specific regulatory considerations for the development of a drug that reduces, mitigates, prevents, or treats noise-induced hearing loss (NIHL) are reviewed, including both preclinical (Sect. 5.2) and clinical (Sects. 5.3 and 5.4) development, followed by discussion of the investigational drugs that are furthest along in clinical testing (Sect. 5.5). Readers well versed in preclinical test methodology may choose to go directly to Sect. 5.3. Section 5.2 highlights issues in developing preclinical datasets prior to human testing.

5.2 Preclinical Efficacy: Designing "Proof-of-Mechanism" Studies with Translational Value

There are multiple variables in the design of preclinical efficacy assessments for NIHL prevention and treatment. A key issue in NIHL research is the lack of standardized animal models or protocols for the evaluation of lead or candidate compounds, which reduces the ability to make direct efficacy comparisons between different compounds. Effective preclinical disease models should closely replicate the human disease in both pathology and physiology and involve routes of drug

administration or treatments that translate directly to their intended clinical application or indication. Animal models for NIHL are numerous. The adoption of common models with respect to experimental variables including noise exposure (Sect. 5.2.1), species selection (Sect. 5.2.2), route of administration and dose–response testing (Sect. 5.2.3), and auditory assessments (Sect. 5.2.4) are necessary to compare lead compounds for their relative safety and efficacy.

5.2.1 *"Replication" of the Human Disease: Laboratory Sound Exposures*

In daily life, individuals are exposed to different types of noise based on their occupation, recreational interests, and other everyday activities. Therefore, in defining noise exposures for use in preclinical studies, investigators need to determine the target population for which they want to model relevant noise exposures. NIHL has two phases: a temporary threshold shift (TTS) hearing loss following a noise exposure and a permanent threshold shift (PTS) hearing loss that does not resolve to baseline after a noise exposure. Although there is some debate over when hearing loss should be defined as permanent, there is generally good agreement that most TTS will resolve within the first 14 days, and by 30 days postnoise, any remaining threshold shift is a PTS. Investigators must select a level and duration of noise to elicit a desired TTS or PTS based on their population to be modeled (Hu 2012). In addition to intensity and duration, spectral characteristics of the exposure, such as whether the exposure is an impulse noise or a continuous noise, may be important (Henderson and Hamernik 2012). The potential for repeat insults within the human population of interest should also be considered as part of the animal model.

Beyond the basic selection of broadband, octave band, narrowband, or impulsive noise, investigators need to select a method of delivery for the noise insult. Important considerations include free field versus closed field, binaural versus monaural, restrained versus unrestrained, and awake versus anesthetized animals. Unfortunately, anesthetics have the potential to alter the pharmacology and toxicology of otoprotective compounds or drugs. In addition, the anesthesia used to immobilize the animal before and during the noise exposure may alter the animal's response to the noise trauma (Chung et al. 2007).

Noise exposure paradigms often vary across laboratories, making comparisons difficult, if not impossible. Common noise exposures for preclinical testing among different laboratories would facilitate drug comparisons (for detailed discussion, see Le Prell and Miller 2016). That said, because human noise exposures outside the laboratory are highly variable from one setting to another, there is a need for multiple agreed-on protocols, including broadband, octave-band, and impulse noise exposures at a minimum and narrowband and pure-tone exposure as an option. The issue of TTS and PTS within these noise protocols needs careful consideration,

given that both are clinically relevant and may involve similar or disparate drug targets.

5.2.2 *Species Commonly Used in Otoprotection Research*

Rodents are the most commonly used laboratory models in preclinical NIHL studies. Here, various mouse and rat strains, *Cavia porcellus* (guinea pig), and *Chinchilla lanigera* (chinchilla) are briefly discussed. In any model, it is preferable to work with specific pathogen-free (SPF) organisms from qualified commercial vendors to reduce the potential for subclinical diseases or conditions that may confound experimental results.

5.2.2.1 Mouse Models

Mice are relatively easy to breed and easily housed in large numbers, and a wide variety of genetically modified strains are commercially available. However, mice can be difficult to dose orally and often require significantly higher drug doses than other rodents based on their very rapid metabolism. Owing to their small size, small deviations in dosing volume tend to impact both pharmacokinetic (Pk) and pharmacodynamic (PD) values. In addition, mice have an "audiogram" or range of hearing that is shifted to a higher frequency range than humans. Noise exposure paradigms in mouse studies typically include extended high frequencies (i.e., >8 kHz), largely above the range considered critical for human hearing. Auditory function has been documented in a large number of inbred mouse strains (Zheng et al. 1999). Some of the most common mouse strains used in NIHL studies are the CBA/Ca and CBA/CaJ. Another common strain is the C57BL/6J, although this strain carries a Cadherin 23 (Cdh23) mutation that dramatically influences the age of onset and rate of progression of hearing loss across the life span. Age is an important factor, as mice exhibit an increased sensitivity to noise in the first 4–8 weeks of life (Kujawa and Liberman 2006), with vulnerability decreasing across the remaining life span (Henry 1982). Interestingly, these differences vary across strains (Li et al. 1993) and some strains appear more resistant to NIHL than others. These differences raise important questions about which is the "best" strain to use to model human NIHL, in addition to questions about whether mice are the "best" rodent species as a whole given differences in hearing and metabolism.

5.2.2.2 Rat Models

Relative to mice, the rat's larger size makes certain aspects of auditory testing and drug dosing easier to accomplish. Consequently, rats are popular in hearing research, and auditory thresholds have been reported across a number of strains

(Borg 1982). Some of the most commonly used strains for NIHL research are Sprague-Dawley, Long-Evans, and Fischer 344. This again raises questions about which is the "best" strain and "best" species. Like mice, rats are more sensitive to higher frequency sounds than humans and exhibit increased sensitivity to noise in the first 6 weeks after hearing onset (Lenoir et al. 1979).

5.2.2.3 Guinea Pig Models

The use of guinea pigs as laboratory animals is highly regulated by the US Department of Agriculture (USDA). As a "covered species," there are additional considerations regarding their acquisition, monitoring, and reporting of their use for biomedical research (National Research Council 1996). Sourcing of SPF guinea pigs can be problematic because of endemic cytomegalovirus (a leading cause of unilateral SNHL) in many commercially available colonies, but generation of SPF colonies is possible. Guinea pig breeding is also more challenging owing to lower fecundity and longer gestational periods relative to mice and rats (Ediger 1976).

Despite the aforementioned challenges, there are multiple advantages to using guinea pigs for studies on NIHL otoprotection. First, guinea pigs have better low-frequency hearing than most mice and rats (4–8 kHz). Second, guinea pigs have significantly larger mastoid cavities or bullae and cochlear volumes than mice or rats, making local or inner ear drug delivery possible. Drugs and investigational compounds can be delivered directly to the cochlea via a cochleostomy (a hole drilled through the bony wall of the cochlea) or through the round window membrane (RWM). RWM application techniques include acute topical applications (Lemke et al. 2009), surgical implantation of an infusion cannula (Brown et al. 1993; Miller et al. 2007), and drug-diffusing gels such as Gelfoam® (Lemke et al. 2009; Eshraghi et al. 2013). Because differences exist in the noise vulnerability of albino versus pigmented animals (Pye 1987), strains must still be selected carefully with respect to "best" modeling of the human condition of interest.

5.2.2.4 Chinchilla

The final rodent species to be discussed here is chinchilla, a species also covered by the USDA. Chinchillas have a long history of use in auditory research because their range of hearing is similar to that of humans, with increased hearing sensitivity and noise vulnerability at 4 kHz. Like the guinea pig, the cochlea and round window can be readily visualized for local or inner ear drug administration. However, SPF sourcing, vivarium housing, and oral dosing considerations place them in line with guinea pigs as one of the more challenging species to work with from a technical and regulatory perspective. In addition, the pharmacology (Pk and PD) of many approved and investigational drugs are not known in chinchilla, as they are not widely used in studies beyond the peripheral auditory system.

5.2.3 Route and Timing of Administration

Injection (subcutaneous, intramuscular, or intraperitoneal) is the common route of administration for drugs in most animal studies. Although oral dosing as a route of administration has not been widely adopted in otoprotective studies, translation to human clinical trials and ultimate approval will likely require oral administration. Oral gavage is more time consuming and technically challenging than injection and can result in trauma to the oropharynx and inhalation of the drug into the lungs. Some studies have instead delivered compounds in the animal's food (Le Prell et al. 2011a, 2014) or water (Ojano-Dirain et al. 2013); however, this results in uncertain dosing and Pk/PD parameters because dietary consumption is difficult to measure accurately, particularly in the case of multiple animals housed in the same cage. Even with single-animal housing, water bottles may leak or chow might be removed from the dispenser without being consumed.

In addition to dosing methodology differences across studies, there are significant differences with respect to the start of dosing. The start of dosing might be hours or days before the noise exposure, and once begun, dosing might continue for hours, days, weeks, or even months after the noise exposure. Although some studies do include postnoise treatment dosing, dosing in most otoprotective animal studies begins prenoise to establish steady-state Pk. It is unclear whether this dosing strategy will be effective in pivotal or Phase 3 clinical trials that are required for drug product registration or a new drug application (NDA). Perhaps the most important issue with respect to dosing, however, is the failure of most preclinical investigations to establish a dose response or lack of dose response, as would be expected for any drug development effort (Spruill et al. 2014).

The route of administration of a drug can significantly impact its safety and efficacy profile as well as its potential marketability. For the prevention or treatment of occupational NIHL, oral delivery will likely be required. Oral administration is one of the least invasive and easiest methods of drug delivery for humans, particularly for chronic indications or treatment. If a drug is limited by moderate or potentially severe adverse events (AEs) or side effects, then local delivery by intratympanic injection (ITI) may be more favorable. However, this route of administration has its own side effect profile, including pain, perforation, and infection, and requires a trained physician or otolaryngologist to administer. In a single-center study involving 11 subjects, the drug AM-111 was administered by ITI postnoise exposure for the treatment of acute acoustic trauma (Suckfuell et al. 2007). Here, 13 AEs were reported in 5 subjects. Similar side effects have also been reported in larger studies using ITI drug administration for other indications including local steroid treatment after idiopathic sudden hearing loss (Rauch et al. 2011) or ITI dexamethasone (OTO-104, a sustained release dexamethasone hydrogel) to treat Ménière's disease (Lambert et al. 2012). Intravenous (IV) administration may be an alternative to oral delivery or ITI administration for drugs with poor oral bioavailability or where ITI is not possible. Although there do not appear to be any well-controlled IV-based clinical trials involving NIHL, IV

administration of drugs has been used after sudden sensorineural hearing loss (Mora et al. 2003; Kang et al. 2013).

5.2.4 Auditory Assessments in Preclinical Models

The auditory brainstem response (ABR) and otoacoustic emissions (OAEs) are the most commonly collected data in preclinical tests, whereas human studies typically assess pure-tone threshold sensitivity behaviorally and perhaps include OAEs. Brief descriptions of these and other metrics are described in Sects. 5.2.4.1–5.2.4.6.

5.2.4.1 Auditory Brainstem Response

Prevention of TTS and PTS in preclinical models is most commonly assessed using the acoustically evoked ABR threshold. The ABR is a tone or click-evoked synchronized neural response to calibrated sounds such as tone pips or clicks. The evoked activity along the ascending auditory pathway is recorded in humans using electrodes placed on the scalp and earlobe or mastoid, and it is recorded in anesthetized animals using subcutaneous electrodes. The specific test frequencies vary as a function of species (e.g., chinchillas and guinea pigs have lower frequency audiograms than rats or mice and are therefore tested at lower frequencies). Although ABR threshold testing is not commonly used for the diagnosis or monitoring of NIHL in humans, it is widely used in both preclinical and clinical settings. It would therefore be beneficial to develop a common preclinical ABR testing methodology to allow comparisons among studies and across compounds.

In addition to the common threshold metric, amplitude has been suggested as an important new clinical test metric (for review, see Kujawa and Liberman 2015), but specific clinical deficits due to decreased ABR wave 1 amplitudes have not been shown. Therefore, several clinical studies are needed before this method becomes an adopted clinical end point (Le Prell and Lobarinas 2015; Le Prell and Brungart, in press). Although the ABR could be used to define specific thresholds and threshold shifts clinically, there is a significant challenge to using ABR threshold testing in humans. Specifically, there is a much greater noise background that arises in part because humans are tested in an awake state to avoid anesthesia-related complications and cost as well as the increased noise from the greater distance between the scalp electrode and the brainstem generator in human heads relative to laboratory rodents.

5.2.4.2 Otoacoustic Emissions

Outer hair cell (OHC) function is routinely inferred using OAEs. OAEs are sounds recorded in the ear canal via a microphone that are generated by nonlinearities

produced by OHCs that are reliable correlates of inner ear health (Kemp 2008). All vertebrates studied to date are capable of generating some level of OAE either spontaneously [spontaneous otoacoustic emission (SOAE)], evoked by a transient sound [transient evoked otoacoustic emission (TEOAE)], or evoked by two tones and measured at a different distortion frequency [distortion product otoacoustic emission (DPOAE)] (mammals, see Lonsbury-Martin and Martin 2008; amphibians, see Manley and Van Dijk 2008). Despite the variety of cochlear shapes and mechanics present across the broad class of vertebrates studied, the widespread existence of OAEs suggests a common mechanism among vertebrates likely associated with the amplification and fine tuning of the auditory system for optimal sensitivity (Bergevin et al. 2015).

Although not a test of hearing, changes in DPOAE amplitudes have been proposed as an early identifier of NIHL, with potential application in occupational noise-monitoring programs (Konrad-Martin et al. 2012). OAE metrics may reveal damaged OHCs in the absence of overt hearing loss, but the utility of OAE measurements as a metric for noise-induced OHC damage in clinical trials will require additional validation studies before this measure can become a routine aspect of drug development. As such, although helpful in understanding the pathology of hearing loss, OAEs will probably remain an exploratory or secondary clinical end point (for discussion, see Le Prell and Lobarinas 2015). In summary, OAE measurements are now quite common and routinely supplement pure-tone audiometry in published works, but pure-tone audiometry remains the gold standard for clinical testing and the determination and progression of acute and chronic NIHL.

5.2.4.3 Behavioral Audiometry

Because ABR thresholds closely match behaviorally derived thresholds in mammals (Le Prell et al. 2004), there is often little incentive for investigators to undertake the time and expense of training animals to perform an operant response for reporting the detection or discrimination of different features of acoustic signals. Trained behaviors are usually maintained using positive reinforcement such as food or water for correct responses or by shock avoidance paradigms where animals are punished with a transient foot shock for failing to make correct responses. The training time required, and the increased costs associated with the long-term care and personnel time, generally reduce enthusiasm for this approach. This comment should *not* be taken to suggest there is no utility in operant experiments. Psychophysical investigations using operant conditioning have been used to measure frequency selectivity (Serafin et al. 1982; Prosen et al. 1989), sensitivity to intensity changes (Prosen et al. 1981; Le Prell et al. 2001), amplitude modulation (Moody 1994), formant frequency (Sommers et al. 1992), phase (Moody et al. 1998), rise time (Prosen and Moody 1995), and masking (Le Prell et al. 2006). Animals can also be trained to explore other phenomena such as categorical perception (Kuhl 1986; May et al. 1989). Collection of these measures may be

increasingly important in the future given suggestions that noise insult results in suprathreshold processing deficits that are "hidden" in an otherwise normal audiogram (for review, see Kujawa and Liberman 2015).

5.2.4.4 Audiometry Using Suppression of Reflexes

An alternative to operant tasks is the use of acoustic signals to mediate reflexive responses. For example, the acoustic startle reflex is a whole body motor response to unexpected high-level sound present in both animals and humans. This response can be attenuated by presenting a lower level "cue" stimulus before the louder startle stimulus, a phenomenon termed prepulse inhibition (PPI). PPI has been used to generate "audiograms" with threshold estimated based on the minimum sound levels at which prepulse signals effectively inhibit the acoustic startle reflex (Ison et al. 2002; Tziridis et al. 2012). Prepulse signals that are inaudible should not suppress the acoustic startle response. Detailed discussion of the acoustic startle reflex in auditory tests is provided by Allman, Schormans, Typlt, and Lobarinas in Chap. 7.

5.2.4.5 Otoscopy and Tympanometry

During otoscopy, the ear canal is inspected and the tympanic membrane is visualized. Tympanometry then provides measurements of the mobility and impedance of the tympanic membrane and middle ear ossicles. This validated measure is not a viable end point but is frequently included as part of the screening criteria in NIHL studies, with individuals who have conductive or mixed hearing loss excluded. For preclinical studies, otoscopic evaluation of the animal is sometimes completed, but tympanometry is rarely performed. Protocols are available for guinea pig (Darrouzet et al. 2007; Dai and Gan 2008), chinchilla (Margolis et al. 2001; Akinpelu et al. 2015), rat (Popelar et al. 2003; Bielefeld et al. 2008), and even mouse (Zheng et al. 2007). Because changes in tympanic membrane compliance can account for some individual variability observed between animal subjects, tympanometric testing may be worth including in preclinical investigations.

5.2.4.6 Tinnitus Tests

A variety of issues have made it difficult to study subjective tinnitus in animals; correct reinforcement or punishment for sound reporting responses in animals that *might* have experimentally induced tinnitus are particularly problematic as the investigator has no a priori knowledge regarding which animals develop tinnitus or how severe an individual animal's tinnitus might be (Moody 2004). A new paradigm for the identification of tinnitus has emerged that involves a modification of the PPI paradigm (Turner et al. 2006). Instead of using an acoustic PPI cue in a

silent background, a silent gap in a continuous background noise is used to modulate the acoustically evoked startle response. Presumably, when the background noise is similar to the animal's tinnitus spectrum, the animal cannot reliably detect the silent gap, and the gap fails to modulate the startle response. This loss of gap prepulse inhibition as a measure of tinnitus has been demonstrated in both salicylate- (Yang et al. 2007) and noise- (Longenecker and Galazyuk 2011; Nowotny et al. 2011) induced tinnitus models. Use of gap detection for tinnitus has not been validated clinically and has not been used in NIHL clinical studies or trials; therefore it is not discussed further here. Use of this test is discussed in detail by Allman, Schormans, Typlt, and Lobarinas in Chap. 7.

5.2.5 *Histological Assessments to Elucidate Mechanisms of Protection*

Preclinical evaluation of the effects of noise on the mammalian cochlea have generally focused on OHC damage or loss, swelling of the stria vascularis, afferent dendrite swelling, and the loss of presynaptic and postsynaptic elements between the inner hair cell and auditory nerve. These measurements require postmortem collection of cochlear tissues and significant technical expertise in the subsequent processing and analysis. Histologic evaluation of the human cochlea is not generally performed except in cases where temporal bones have been donated for medical research (as in Makary et al. 2011, for example). In preclinical studies, histologic analysis can reveal the site or mechanism of action of the drug and further elucidate what audiometric assessment may best reveal a physiologic change in human studies (DPOAE, electrocochleography, or ABR). The timing and methods chosen for sample collection, preservation, and processing substantively affect histological quality, and protocols should be developed in consultation with the relevant literature.

5.2.6 *Summary of Preclinical Testing Issues in Translational Investigations*

It would be beneficial to the field if agreement on common animal models were achieved. The diversity of models emphasized by different laboratories developing individual agents has made comparisons of relative efficacy difficult. Appropriate rationales for selecting specific species for otoprotection research should include their similarities to humans both in terms of auditory function as well as in pharmacology and pharmacodynamics of the drug of interest (see Sect. 5.3). From a drug development perspective, it is problematic that there is no agreement on either the most appropriate animal species or most effective noise exposure in translational

auditory research. To speed the development of promising drugs, standardization of the multiple variables in the design of preclinical NIHL studies is needed. In Sect. 5.3, translation of an investigational new drug from preclinical assessment into human testing is discussed. An IND (Sect. 5.3) is required to proceed with clinical studies, which are identified as falling into four stages (Sect. 5.4).

5.3 The Investigational New Drug Application

5.3.1 Pharmacokinetic Assessment

Pharmacokinetics (Pk) is the study of the time course of drug absorption, distribution, metabolism, and excretion (ADME) whereas pharmacodynamics (PD) refers to the effect of the drug on the body and is often determined by a change in a circulating biomarker (Spruill et al. 2014). Ultimately Pk and PD information is used to optimize dose, dose schedule, and the relationship to an efficacy end point in pivotal studies (see Fig. 5.2). Pk and PD information can drive modification of chemical structure to optimize drug activity. NCEs are often developed from parent or lead molecules that have been chemically altered based on their structure–activity relationship (SAR), ADME, Pk, or PD response observed in earlier studies. Basic scientists rarely have training in SAR, ADME, Pk, or PD tests, but they are an integral part of progressing a new agent from animal testing to first-in-man (FIM) studies.

In addition to Pk and PD, there needs to be an early assessment of toxicity, which is generally performed in mice. However, it is also important to select an animal species that metabolizes the drug similarly to humans. The FDA requires the selection of a nonrodent species such as a dog, minipig, or monkey that best represents the ADME, Pk and PD of the drug in humans for all in vivo toxicology or toxicokinetic studies. Metabolic profiling is critical to the IND process because the drug's metabolite may exert important biologic activity and affect both the drug's safety and efficacy. When a compound successfully "passes" early in vitro and in vivo assessments, there is still significant work that must be done to establish appropriate manufacturing procedures.

5.3.2 Chemistry, Manufacturing, and Controls

When a candidate compound is selected for further investigation, efforts to establish the most efficient route of synthesis in terms of cost, yield, and impurities will be made. This becomes of particular concern if exotic intermediates are required for the synthesis, as they may be difficult or impossible to obtain at commercial scale.

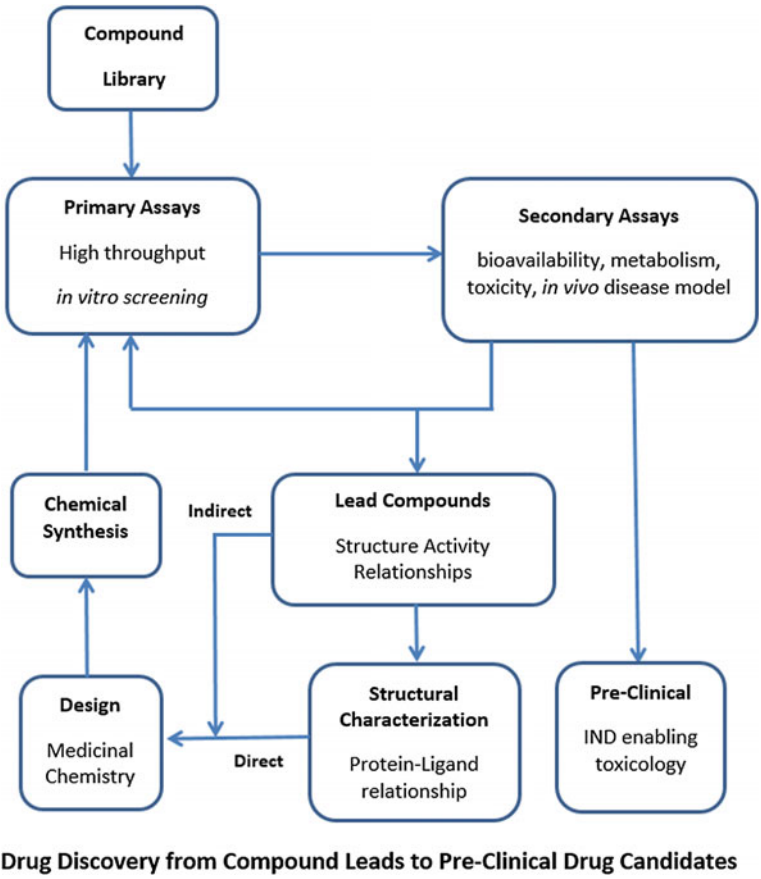

Fig. 5.2 Drug discovery: the preclinical activities required in the translation of an agent from a library of compounds into an investigational new drug candidate. After identification of an agent of interest, a series of in vitro and in vivo tests are required to screen both safety and efficacy. From these assays, candidates emerge and go through further development to understand better the chemistry of the agent and biochemical effects, and to design chemical formulations that are bioavailable, with each new chemical iteration undergoing new assays for safety and efficacy. Completion of this process is achieved when data are adequate to allow approval of an Investigational New Drug (IND) application to the FDA, for first-in-man studies. Results of those studies may drive further iterations of the process illustrated here (Adapted from https://commons.wikimedia.org/wiki/File:Drug_discovery_cycle.svg)

Medicinal chemists provide significant developmental input at this stage; their expertise allows modification of the compound to improve stability and solubility as well as increase scalability for manufacturing. It may be necessary to establish an early-stage reference supply of the compound for comparison to subsequent batches generated by the same or alternative synthesis routes. After the major metabolites are identified, these need to be synthesized for use as a reference material for subsequent bioanalysis of the parent and metabolites in IND-enabling toxicology

studies and later in clinical studies. Methods for determining the identity and purity of the parent compound and associated impurities must be developed and validated for use on subsequent batches analyzed in IND-enabling toxicology studies. The ultimate goal of these activities is to optimize the manufacturing protocols, determine all materials (metabolites) that need to be assessed for safety, and define the release specifications for the drug substance and drug product (see Fig. 5.2). All of these tests are required to be performed using good laboratory practice (GLP), which is a set of standards that ensure consistency, reliability, and reproducibility of the data through uniform, validated procedures completed with calibrated equipment.

5.3.3 IND-Enabling Toxicology

Questions that lead to a thorough understanding of the mechanism of action, possible side effects, dose-limiting toxicities, route of administration, drug half-life, drug metabolites, elimination routes, and possible drug–drug interactions must be asked and adequately answered after preclinical efficacy has been established. A major goal of the IND-enabling toxicology is to determine a recommended dose and schedule for a FIM clinical safety study. An essential component of the IND package will include in vitro and in vivo mutagenicity and carcinogenicity studies. These are typically in the form of an Ames test in bacteria for mutagenicity, a chromosomal aberration test in cultured cell lines, and a micronucleus test in vivo. These requirements may change based on past history of testing of the components in the NCE, given the known risk factors. As availability of the new candidate drug may be limited during early development and characterization steps, a number of contract research organizations (CROs) also offer non-GLP microversions of these tests using smaller amounts of the NCE. The much more costly GLP studies require larger volumes of the active pharmaceutical ingredient (API).

When assessing an NCE in toxicology studies, impurities should be present at a level above what is present in the final marketed drug product to ensure adequate testing of the impurities. In general, during process development, the impurity levels are reduced with manufacturing refinements. However, when scaling up to commercial batch sizes, these improvements may be lost and impurity levels can increase. Depending on the complexity of the synthesis and cost of the starting raw materials or intermediates, the price for manufacturing of the drug substance under good manufacturing practice (GMP), and the development and validation of test methods to characterize the drug substance, run into the hundreds of thousands of dollars for production of sufficient qualified material appropriate for the preclinical toxicology studies. These figures can be increased dramatically for biologics or compounds with extraordinary synthesis routes.

Depending on the proposed route of administration and duration of exposure, the requirements for IND-enabling toxicology may vary significantly. For most drugs given orally, FDA will generally require at least two routes of administration be

evaluated for acute administration. Typically, these are oral and intravenous acute toxicity studies performed in one rodent and one nonrodent species. In these studies, it is imperative that exposure to impurities and metabolites be sufficient to identify toxicity signals in the species chosen in order to be considered reasonably predictive of human toxicity. If multiple doses in human clinical studies are predicted or proposed, chronic toxicity studies of a duration equal to or preferably greater than the exposure duration in human trials will be required in generally one rodent and one nonrodent species. For all of these studies, careful attention to and documentation of efforts to improve the formulation chosen for delivery to optimize exposure in the test animals need to be included. Frequently, non-GLP pilot studies are performed to evaluate potential formulations and perform dose–response assessments before performing costly and time-intensive GLP toxicology studies. Pilot studies may ensure that the toxicology studies performed under GLP are able to identify dose-limiting toxicities and/or establish maximum feasible dose criteria for the studies before their performance. Consultation with a regulatory toxicology expert to plan and perform an acceptable IND toxicology program is highly recommended.

5.3.4 Filing an IND

5.3.4.1 eCTD Format

Current regulatory filing with the FDA is performed using the "electronic Common Technical Document" (eCTD) format developed by the ICH Multidisciplinary Group 2 Expert Working Group (ICH M2 EWG). The eCTD is a hierarchical HTML electronic structure developed to assist in the transfer of regulatory information to the FDA. Before submission of an IND in eCTD format, a sponsor is required to contact the e-submission support team and arrange to submit a sample eCTD or standardized data set. On review and approval of the validated structure, you are then invited to submit an eCTD IND. There is extensive documentation on the FDA website to guide in the development and filing of an eCTD IND. In general, specialized software packages are very helpful to address the regulatory requirements of eCTD filing, submission, and maintenance. It is of course possible that regulatory filing procedures or software requirements will change over time and therefore it is highly recommended to work with or engage a regulatory expert or group familiar with the eCTD process to accelerate submissions and ensure adherence to current FDA and ICH guidance.

5.3.4.2 Indication for Use

Determining the indication for use for a drug to prevent and/or treat NIHL is challenging in that this remains an area with no currently approved drugs and thus

no marketed products with approved labels and indications. Typically, indications for use are constrained by acute, intermittent, or chronic use. For NIHL, acute or intermittent use may be appropriate if it is related to treatment at or near the time of the acute noise exposure or injury. However, for workers exposed to hazardous noise on a daily basis, chronic daily treatment across an occupational career may be indicated. A decision on the intended use of the drug product should be made early on. Although a target product profile (TPP) is not required at the filing of an IND, a draft TPP should be created early in the development process. The TPP is typically the goal of an end of Phase 2 meeting with the FDA. Major modifications to an indication (e.g., change in target population) may require the filing of a new IND application, especially if the disease is covered by a different division of the FDA. An example of the components of a TPP for a drug that prevents or treats NIHL is provided in Table 5.1.

5.3.4.3 FDA Division

Currently, the Division of Neurology Drug Products (DNP) at the FDA has been involved in the filing, review, allowance, and oversight of most, if not all, clinical trials for NIHL. Over the last decade, the DNP has gained significant experience in working with companies seeking to develop drugs for the prevention and treatment of NIHL as well as other acquired forms of SNHL. As of the writing of this chapter, there are no drugs that have been approved by the FDA for the prevention or treatment of any SNHL disease or disorder, let alone NIHL. As NIHL is a novel therapeutic indication with no marketed products, DNP is playing a significant role in reviewing the criteria for early-, middle-, and late-stage clinical studies. DNP has established a liaison with the DoD Hearing Center of Excellence to learn about audiology and otology. In general, the medical device industry for hearing loss is much more advanced because of the decades of work in approving hearing aids and cochlear implants in adults and children. It is not uncommon for review teams within the DNP to consult with their colleagues in the Center for Devices and Radiologic Health (CDRH) that contains a Division of Ear, Nose, and Throat (ENT) devices.

5.4 Clinical Safety and Efficacy

5.4.1 Phase 1 Studies

Phase 1 studies include FIM safety and pharmacokinetic tests of the investigational drug. They are intended to determine the tolerability, dosing, and AEs or side effects associated with dosing of the drug. Phase 1 studies can be randomized controlled trials (RCTs) that include a placebo comparator and blinding to the

Table 5.1 Sample target product profile (TPP)

Product description/mechanism of action	Small molecule, hormone, peptide, or antibody by oral delivery or injection. Drug X increases the activity of an inner ear antioxidant enzyme critical to hearing function
Indication	Prevention and treatment of NIHL
Efficacy end points	Reduction of NIHL using pure-tone audiometry
Primary efficacy end point	Reduction in the magnitude of the TTS/PTS (dB HL)
Secondary efficacy end point	Reduction in the loss of word recognition scores (WRS)
Clinical assessment of success	Clinically relevant improvement in HL by ≥ 25 %
Contraindications	List situations in which the drug should not be used because the known risk outweighs any potential benefit. Known hazards from clinical studies. If no known contraindications exist, state "none known"
Warnings	List serious drug-related adverse reactions from clinical studies and potential safety hazards. Describe the preclinical and clinical data that indicate the product's potential carcinogenic, mutagenic, and/or fertility side effects
Precautions General Carcinogenesis Mutagenesis Impairment of fertility Pregnancy Nursing mothers	Describe any special care to be exercised by the physician or patient for the safe and effective use of the product, e.g., precautions not listed under any other section. Describe the animal studies performed to evaluate the pharmacology, toxicology, carcinogenicity, mutagenicity, and reproductive toxicities. If no nonclinical studies have been done, state this Identify Pregnancy Category A, B, C, D or X. If no nonclinical studies have been done, state this List information about excretion of the drug in human milk and the effects on the nursing infant. If no nonclinical studies have been done, state this
Adverse reactions	List drug-related adverse events seen in clinical trials or anticipated from preclinical pharmacology and toxicology
Dosage and administration/market size	List recommended route (oral, IV, transtympanic, inhaled), regimen (e.g., twice daily), dosage (mg), and duration of treatment (e.g., 10 days). US market size for acute and chronic NIHL could be 10–30 million adults
How supplied	Describe product and administration configuration

The TPP is a living document that is good to start at the beginning of a drug development program and to update after each completed phase of development and as more information is accumulated on the drug's safety and efficacy as well as any changes to the clinical indication, intended use or specific population, packaging, and labeling [Adapted from Scannon (2012); see also http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm080593.pdf]

subject and clinician or observer. Single ascending doses will typically start in a healthy adult population. Multiple ascending doses are typically started in healthy volunteers and then extended to subjects with the disease, especially if the elimination or clearance of the drug is altered by the disease and/or the intended Phase 2

population has impaired renal or hepatic function. Multidose testing is also performed to establish steady-state kinetics for multiday dosing protocols and involves a series of peak and trough plasma measurements to determine the concentration of the drug and its major and minor metabolites. PD assessments can also be addressed in Phase 1 studies, especially if a potential AE involves a drug–drug interaction or the intended Phase 2 clinical end point is a PD assessment. PD assessments are often measured during and after the treatment period to determine if any beneficial change in a biomarker can be elicited. While the FDA does not differentiate between Phase 1a and Phase 1b studies, the pharmaceutical industry typically designates Phase 1 studies in a diseased population as a Phase 1b study. The remainder of this section describes a case study in the form of SPI-1005.

5.4.1.1 Safety Assessments for SPI-1005

Lynch and Kil (2009) describe a Phase 1 clinical trial with ebselen (SPI-1005), an investigational new drug for the prevention and treatment of NIHL, in a randomized double-blind placebo-controlled single ascending-dose study. Thirty-two normal healthy subjects were consented, screened, and enrolled at a single clinical site (MDS Pharma, Lincoln, Nebraska) for a 72-h inpatient stay. History and physical (H&P) examinations, vital signs including orthostatic measurements, 12-lead electrocardiograms (ECGs), hematology [complete blood count (CBC) with differential], serology (Chemistry-20, also termed Chem-20, a complete chemistry screen that measures 20 different substances), and urinalysis were repeated over a 2-week period. AE monitoring was conducted repeatedly over the 3-day inpatient period. This study was completed and published in a peer-reviewed scientific journal. There is no requirement for Phase 1 studies to be published or listed on clinicaltrials.gov but as per the introductory comments, release of data in a peer-reviewed format may facilitate development and acceptance as well as driving new preclinical assessment of investigational drugs in other laboratories. The test battery is listed in detail here to highlight the multiple components monitored within a Phase 1 study as part of initial safety assessments.

5.4.2 Pharmacokinetics

A key component of Phase 1 testing is Pk assessment. As part of this Phase 1 study, Lynch and Kil (2009) reported data on peak plasma concentration (C_{max}), the time after ingesting the drug that it takes to reach maximal concentration (T_{max}), and half-life ($t_{1/2}$), which is the time it takes for 50 % of the drug to be eliminated from plasma. Finally, the total concentration or exposure (AUC_{0-t}) for ebselen and its three metabolites were calculated, as well as the plasma selenium concentration. Data generated in this Phase 1 study were a necessary step in the drug development process, ultimately setting the stage for a Phase 2 study assessing safety and efficacy

of SPI-1005 (NCT01444846; Kil et al. 2014). Details are shared here to illustrate the kinds of data that are sought in preclinical and early Phase 1 clinical testing for an NCE.

Critical to the discussions with the FDA is a robust review of any AEs at each dose level tested. This is essential in guiding the acceptable dose range and schedule and the potential allowance of additional clinical studies. If multidose schedules are proposed in future studies, supporting toxicology studies performed in appropriate species over a duration of time equal to or greater than the clinical dosing schedule need to be provided and reviewed. The data gathered in Phase 1 studies allow sponsors to make an informed decision on what dose ranges are well tolerated and guide establishment of a risk-to-benefit ratio in relationship to the pursued indication. In the case of the SPI-1005 Phase 1 data, with no AEs reported at all doses tested (single oral dose up to 1,600 mg) but no multidose testing, approval for multidose Phase 2 studies relied on preclinical toxicology studies run in multiple species for 28 days.

5.4.3 Phase 2 Studies

Phase 2 studies include larger numbers of subjects than Phase 1 studies and are intended to provide an initial assessment of efficacy in the affected population. They also further assess safety in the affected population. This section introduces the Phase 2 clinical trial of SPI-1005 in the prevention of NIHL (NCT01444846).

5.4.3.1 Trial Design

This single-center study recruited healthy volunteers 18–31 years of age at the University of Florida; enrollment was completed in November 2013. This was a dose-escalating design with three treatment arms. Participants in Arm 1 were randomized to take oral SPI-1005 at a dose of 200 mg twice daily or matching placebo. Participants in Arm 2 were randomized to take oral SPI-1005 at a dose of 400 mg twice daily or matching placebo, and participants in Arm 3 were randomized to take oral SPI-1005 at a dose of 600 mg twice daily or matching placebo (see Fig. 5.3). Dosing was continued over 4 days, starting 2 days before a calibrated sound challenge (CSC) and continuing the day of the CSC and 1 day post-CSC. This was a double-blind design; neither the subjects nor the study team knew the treatment condition of any subject during the course of the study. During the CSC, subjects listened to prerecorded rock or pop music on an iPod® using insert earphones that were set to a specific SPL (averaging 100 dBA in coupler measurements) for a fixed exposure duration (4 h). In a pilot study using this CSC (n = 12), the TTS at 15 min postnoise exposure ranged from 0 to 14 dB, with the group average of 6.3 ± 3.9 dB at 4 kHz (the most affected frequency) (Le Prell et al. 2012).

Pure-tone audiometry was the primary audiometric assessment; TTS was calculated for each tested frequency in each ear over time. The TTS was defined as the difference between the post-sound exposure threshold and the baseline threshold measured immediately before the CSC. Hearing tests were conducted at the time of screening and enrollment (clinic visit 1), immediately before the CSC on the third day of dosing, as well as 15-min, 1-h 15-min, 2-h 15-min, and 3-h 15-min post-CSC (clinic visit 2). Audiometric testing was repeated the following day (clinic visit 3) and 1 week later (clinic visit 4).

5.4.3.2 Medical Monitoring

Subjects had a detailed H&P examination at the time of the screening to determine if they satisfied the inclusion and exclusion criteria. Subjects who passed the H&P and all other laboratory criteria, including audiometry, hematology (CBC), serology (Chemistry-20), and radiology (chest X-ray), were enrolled. The H&P, including

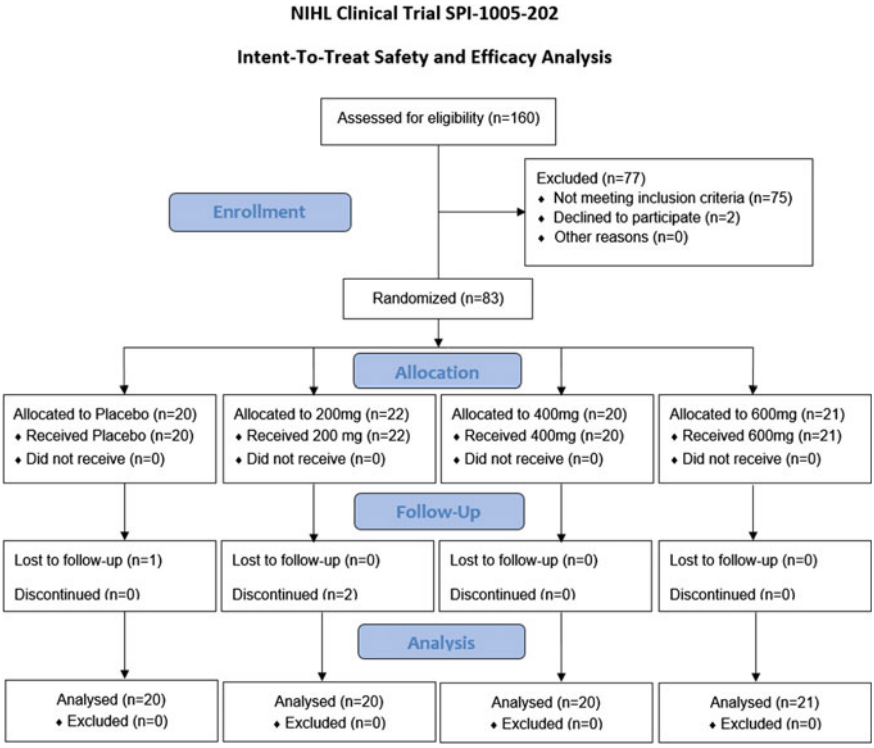

Fig. 5.3 The number of subjects in the Phase 2 clinical trial of SPI-1005 (NCT01451853) are illustrated here. In an intent-to-treat analysis, all subjects are included, regardless of compliance with protocol

Medra queries about AEs, was repeated at clinic visits 2, 3, and 4. Additional blood samples were drawn and all baseline assays repeated at clinic visits 2 and 4. Chest X-ray exams were repeated at clinic visit 4. Details are shared here to illustrate ongoing medical monitoring in Phase 2 studies, akin to that in Phase 1 studies.

5.4.3.3 Pk

Pk assessments are also completed in Phase 2 studies. Here, blood was collected for analysis of plasma ebselen and its metabolites, as well as total plasma selenium, at clinic visit 2. The Pk data from the Phase 1 study with SPI-1005 indicated a plasma $t_{1/2}$ of approximately 6–8 h, suggesting that twice-daily dosing would be appropriate in a multidose protocol. The Pk data also indicated that ebselen reaches C_{max} in approximately 2 h. These data informed decisions for the timing of blood draws to quantify ebselen and its metabolites after the achievement of steady state in Phase 2. In the Phase 2 study, Pk samples were drawn on the morning of the third day of dosing, just prior to the morning dose and 2 h after the morning dose.

Subjects were exposed to the CSC after the second (steady-state) blood sample was drawn. Based on prior preclinical data, efficacy of ebselen is best achieved when dosed at least 1 h before noise exposure (Lynch et al. 2004; Yamasoba et al. 2005). By analyzing Pk samples, exposures to drugs in test subjects can be confirmed and exposure–response relationships can be determined. For studies with multiple dose levels, study sponsors and reviewers can assess whether higher doses of the drug result in higher exposure levels of the drug and metabolites or if there is a saturation effect. One additional parameter that can be evaluated with Pk sampling is confirmation of a subject's compliance with the dosing schedule. One subject assigned to the 600-mg group, who reported compliance with the dosing protocol, showed no detectable levels of ebselen or metabolites in the Pk assessment [using validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method]. In addition, the subject's plasma selenium levels were not elevated above those of placebo subjects and showed no increase from baseline to treatment [using a validated inductively coupled plasma mass spectrometry (ICPMS) method]. These bioanalytic data were not available in real time and were available only several weeks after the study was completed, as is typical for many studies, and, moreover, as required for any double-blind study in which treatment codes are not available until the data are "locked." The study coordinators made every attempt to recall this subject for an interview to discuss this noncompliance issue but did not receive a response. The retained clinical trial material for this subject was sent for analysis, which confirmed the presence of study drug in the remaining capsules at the expected levels. Ultimately, this subject was included in the per protocol efficacy analysis despite strong evidence that the subject was noncompliant with the dosing protocol as there was no conclusive evidence available. Multiple Phase 2 studies are often performed in an effort to adequately define dose range, end points, and power estimates before proceeding with Phase 3 pivotal studies.

5.4.4 Phase 3 Studies: Pivotal Studies

Phase 3 studies often include a large number of subjects. During Phase 3 studies, effectiveness is (hopefully) confirmed, side effects are monitored, and there may be comparisons to other common treatments if there is an established standard of care. The use of a placebo group should be carefully considered in Phase 3 studies, as there are special ethical issues related to care for sick or diseased patients (Hernandez et al. 2014; Keranen et al. 2015; Niemansburg et al. 2015). For the indication of NIHL, a placebo group comparison to active dose group(s) is justifiable given that there are no other currently approved treatments. There is a caveat, however, in that in any study where PTS is expected in the absence of experimental drug intervention, every effort must be made to ensure that all subjects are appropriately educated about the effects of noise on hearing and provided with hearing protective devices as per regulatory agency guidance (OSHA 1983; DoD Instruction 6055.12 2010).

Typically, a NCE for a nonorphan indication will need to be tested in a large number of subjects in two separate pivotal Phase 3 trials, which should result in similar outcomes. Study repetition allows for comparison and evaluation of repeatability by the FDA when submitting a NDA for approval to market a drug for a given indication. In general, exposure to the NCE-containing drug product should include upward of 600 subjects and is commonly composed of 300 drug-exposed subjects in each of two Phase 3 trials. The precise numbers of drug-exposed subjects will be negotiated with the FDA review team and is based on known or potential risks identified in earlier trials as well as power estimates for achieving a statistically robust result on agreed on primary end points for the trial. If the results of the two pivotal Phase 3 trials differ significantly in their outcomes, the FDA may recommend additional confirmatory studies be completed prior to submission of an NDA. A special protocol assessment (SPA) for clinical protocols is a declaration from the FDA that a Phase 3 trial's design is adequate or acceptable for FDA approval. An SPA will seek to reach agreement between the sponsor and the FDA on the pivotal protocol design, end points, safety assessments, and the statistical analysis plan prior to enrollment of the Phase 3 clinical trial.

Given the novelty of NIHL as an indication and the efficacy end points being employed, sponsors may choose to request an SPA by the FDA review team. Clinical protocols for Phase 3 trials where data outcomes form the primary basis for an efficacy claim, if the trials had been the subject of discussion at an end-of-Phase 2/pre-Phase 3 meeting with the review division, may be eligible for an SPA agreement under the Prescription Drug User Fee Act (PDUFA) goals. A request for an SPA must occur before initiation of the pivotal Phase 3 trials. If granted, an SPA agreement can provide an opportunity to get input and buy-in from the FDA on the scientific, regulatory, and statistical rigor of the planned clinical trial. SPAs can also be requested for nonclinical carcinogenicity protocols and CMC stability protocols.

In performing Phase 3 studies, it is imperative that drug developers utilize a drug product consistent with that which will be filed for registration with the FDA. Any

changes in the dose, formulation, form, and manufacturing methods of the drug substance or drug product between the clinical supplies used in the pivotal trials and the drug product in the NDA must be disclosed and could put the approval at risk. If the FDA concludes that the drug product tested in the pivotal trials is not clearly equivalent to what is being proposed for registration or market approval, further studies of the proposed drug product may be required to prove bioequivalence. Typically, a minimum of two registration batches of qualified drug substance and drug product must be manufactured and batch records made available to the FDA to allow adequate review of the processes for use in commercial manufacturing of the drug product.

5.4.5 NDA and Approval

Legal requirements for safety and effectiveness for a drug to be approved have been interpreted as requiring scientific evidence that the benefits of a drug outweigh the risks and that adequate instructions exist for safe use in the intended indication. Well-controlled clinical trials, adequately descriptive labeling and instructions, appropriate and supportive toxicology reports, and well-delineated CMC all are components of a successful NDA approval. Perhaps most interesting to readers of this chapter would be a coherent discussion of the relevant end points for efficacy assessment of an approvable otoprotective drug. The challenge here is that there is no precedence to cite, as there are no approved drugs for the prevention and treatment of NIHL. There are, however, guidance statements from multiple organizations that might prove helpful.

5.4.5.1 Guidance from Professional Societies

Although there is no specific guidance from the FDA as discussed in Sects. 5.3.4.2 and 5.3.4.3, it seems reasonable to predict that noise otoprotection health claims are likely to be based on preservation of threshold sensitivity or perhaps preserved speech test performance given the recommendations of Gurgel et al. (2012). Thresholds are assessed using conventional pure-tone air-conduction audiometric testing using narrowband noises or pure tones as signals. Hearing levels are measured relative to a large reference population; the difference between an individual's measured threshold and the reference threshold is expressed using the units dB HL. Positive numbers reflect poorer hearing relative to the reference population, and negative numbers reflect better hearing than the reference population. In the literature, hearing loss is typically attributed to noise exposure if the audiometric configuration is "notched," meaning that thresholds are poorer at 3, 4, or 6 kHz than at 1 and 2 kHz and 8 kHz. There are a variety of definitions of what constitutes a notched audiogram (for review, see Le Prell et al. 2011b). Two of the more common notch criteria are those of Coles et al. (2000) and Niskar et al. (2001),

requiring either a 10-dB or a 15-dB notch depth, respectively. Hearing loss due to noise is typically the most robust at these 3-, 4-, and 6-kHz frequencies because of the ear canal resonance at approximately 3 kHz and the half-octave shift in which hearing loss is expected at or above the exposure frequency (Ward et al. 2003). If drug benefit is assessed based on some reduction in the prevalence of notched configurations, then the definition of a notch will obviously affect the measured prevalence of audiometric notches in the audiograms from both treated and control populations.

Explicit criteria defining threshold shifts in noise-exposed workers, have been provided by a number of government agencies, including the Occupational Safety and Health Administration (OSHA), the National Institute on Occupational Safety and Health (NIOSH), and the Department of Defense (DoD). OSHA defines a *standard* threshold shift (STS) as "a change in hearing threshold relative to the baseline audiogram of an average of 10 dB or more at 2,000, 3,000, and 4,000 Hz in either ear" [OSHA 1983; Section 1910.95(g)(10)(i)], whereas NIOSH advocates a criterion for *significant* threshold shift (STS) with the recommended definition of change being "an increase of 15 dB in hearing threshold level (HTL) at 500, 1000, 2000, 3000, 4000, or 6000 Hz in either ear, as determined by two consecutive audiometric tests," with the second test required to reduce false-positive findings (NIOSH 1998; p. iv, see also pp. 43–50). The DoD defines STS as "a change in hearing threshold relative to the initial reference audiogram of an average of 10 dB or more at 2,000, 3,000, and 4,000 Hz, in either ear," and they also state, "A single frequency 15 dB shift at 1,000, 2,000, 3,000, or 4,000 Hz is considered an early warning flag with no requirements for follow-up testing or referrals, but with a requirement to counsel the patient and check hearing protection" (DoD Instruction 6055.12 2010). One potential strategy for evaluating otoprotective drug benefit is assessing whether it reduces the percentage of workers, soldiers, or other noise-exposed participants that meet the OSHA, NIOSH, or DoD criteria for an STS. In real-world trials, differential use of hearing protection devices (ear plugs or ear muffs) increases individual variability as it influences individual risk of STS. Campbell and Fox, in Chap. 6, similarly address the issue of how to monitor drug-induced ototoxic hearing loss in clinical trials. They describe ototoxicity criteria put forward by the American Speech-Language-Hearing Association (ASHA). However, just as the ASHA criteria were not designed with the intent to identify benefits of otoprotective agents, the OSHA, NIOSH, and DoD criteria may be relatively insensitive to drug-mediated benefits.

The potential use of criteria that define "compensable hearing loss" might be considered as an alternative, but this is even more problematic in that these criteria are intended to identify individuals who have suffered disabling auditory injury. Guidance from the Veterans Administration (VA) is provided in 38 CFR 3.385, which states, "For the purposes of applying the laws administered by VA, impaired hearing is considered a disability for VA purposes when the auditory threshold in any of the frequencies 500, 1000, 2000, 3000, 4000 Hz is 40 decibels hearing level (dB HL) or greater, the auditory thresholds for at least three of the frequencies 500, 1000, 2000, 3000, or 4000 Hz are 26 dB HL or greater, or speech recognition

scores using the Maryland CNC Test are less than 94 %." The use of speech tests is particularly interesting here, given guidance from the American Academy of Otolaryngology–Head and Neck Surgery (AAO–HNS), which has recommended that word recognition scores be included in *all* clinical trials that assess auditory function (Gurgel et al. 2012). Interest and enthusiasm for speech, and speech-in-noise tasks in particular, appear to be increasing given suggestions that a history of noise exposure may underlie speech-in-noise discrimination difficulties (Kujawa and Liberman 2009; Lin et al. 2011; Makary et al. 2011), but it is not clear at this point what the most sensitive test might be (for detailed discussion, see Le Prell and Lobarinas 2015; Le Prell and Brungart, in press).

5.4.5.2 Guidance from the Department of Defense Hearing Center of Excellence

Recognizing the importance of these kinds of issues, the DoD Hearing Center of Excellence (HCE) has established a Working Group on Pharmaceutical Interventions for Hearing Loss (PIHL). A number of issues discussed in this chapter have been discussed previously in their newsletter series (http://hearing.health.mil/EducationAdvocacy/NewsLetters.aspx), and a series of articles has been prepared with authors from the DoD HCE partnering with academic and industry colleagues to provide a series of guidance documents. Several of these documents have been accepted for publication, with additional articles currently completing the final stages of the review process. These documents are intended to provide best practices and establish common procedures for preclinical and clinical investigation and will be a useful addition to the literature.

5.4.6 Phase 4 Studies: Postmarketing Surveillance Studies

Phase 4 studies are postmarketing surveillance studies, with the potential to expand on the initial label claims approved under the original NDA. These Phase 4 activities are largely outside the scope of this chapter, which is focused on early translational and clinical activities required to achieve of proof of concept in humans.

5.5 Human Clinical Studies in NIHL

Here, brief summaries regarding the development of a subset of the compounds being developed are offered, ending with a few common observations related to the future of these early and midstage exploratory studies.

5.5.1 N-acetylcysteine

N-acetylcysteine (NAC) is the most extensively investigated potentially otoprotective drug to date; its testing was facilitated by several features. First, NAC was already approved by the FDA for acetaminophen overdose and for inhalation as a mucolytic, with an FDA-approved dosing regimen unique to each of these two indications. Thus, there was substantial existing safety data in humans as well as efficacy for a different indication, which is different than developing an NCE for FIM application. Second, NAC received patent protection (Kopke et al. 2003), further facilitating commercial development. NAC was previously marketed as The Hearing Pill® by the American BioHealth Group (Somers 2007; Karlman 2012). In the absence of efficacy data, The Hearing Pill® was promoted as a dietary supplement, or a "nutraceutical," a category that cannot make claims of medical benefit because it was not evaluated by the FDA (Schachtman 2003).

NAC *was* assessed in several human clinical studies, although not in the marketed formulation of The Hearing Pill®. First, Kramer et al. (2006) documented TTS and temporary changes in OAEs in 31 normal-hearing subjects after a single music exposure at a recreational setting (nightclub), with NAC (900 mg) ingested as an effervescent tablet in water 30 min prior to entering a San Diego nightclub. Although no significant otoprotection was observed, the authors suggested that a significant difference in music exposure levels across the eight evenings of data collection (ranging from 92.5 to 102.8 L_{avg}) may have masked potential benefits of NAC. Two additional studies were completed using TTS models. Lin et al. (2010) assessed prevention of noise-induced TTS in 53 male workers at a steel manufacturing facility in Taiwan, reporting an average TTS at 3, 4, and 6 kHz of 2.77 dB after placebo and 2.45 dB after NAC. Although this small difference in TTS was reported to be statistically significant ($p = 0.03$), a 0.3-dB difference is not clinically relevant regardless of statistical reliability. Lindblad et al. (2011) tested NAC in a weapons training setting in which military subjects were exposed to impulse noise, but no reliable TTS was measured, making claims of potential otoprotective benefit impossible.

The methodological issues in these early studies (Kramer et al. 2006; Lin et al. 2010; Lindblad et al. 2011) highlight the importance of having a well-defined trial population and an adequately powered design. A fourth NAC study was more successful in adequately powering the active group against the placebo group. Kopke et al. (2015) assessed threshold shift in 566 normal-hearing participants treated with 900-mg NAC (effervescent tablet in water) or matching placebo (in water) three times daily, for a total of 2700 mg/day during the first 13 days of weapons training, followed by twice daily dosing for 3 days, for a total of 1800 mg/day. During the 16-day weapons training period, Marine recruits were exposed to M-16 rifle fire (325 rounds) and other non-rifle noise, including simulated explosions. Hearing thresholds were determined using 2–16 kHz tonal stimuli, measured before training and 10 days after training had stopped. The primary outcome (which is defined as part of the statistical analysis plan) was defined as the

incidence of a significant threshold shift (STS) including an increase of 20 dB or more at one frequency or 10 dB or more at two adjacent frequencies. There were no significant decreases for the left ear (NAC: 21 % vs. placebo: 19 %, $p = 0.7816$), although the group difference approached $p < 0.05$ for the right ear (NAC: 21 % vs. placebo: 27 %, $p = 0.0562$). Additional comparisons were conducted as secondary analyses; when STS was defined as an increase of 15 dB or more at one frequency or 10 dB or more at two adjacent frequencies, the rate of STS was reliably different for the ear corresponding to the trigger hand (NAC: 27 % vs. placebo: 35 %, $p = 0.0288$) (Kopke et al. 2015). The additional detail provided for this last study is included to highlight the difficulties of clinical trials in which only 19–35 % of the placebo group develop a hearing loss and, moreover, to illustrate the complexity of live fire exercises where the exposure may be asymmetric.

5.5.2 D-Methionine

According to the clinicaltrials.gov website, the study NCT01345474 is currently recruiting subjects from a population of drill sergeant instructor trainees ages 21–40 years at Fort Jackson, South Carolina. Subjects take twice daily oral D-methionine (D-Met) of up to 100 mg/kg/day for 18 days (starting 3 days before training, during 11 days of training, and continuing 4 days posttraining). This is a randomized, double-blind, placebo-controlled clinical trial. During the study, participants are exposed to M-16 fire during weapons training, with a 500-round minimum over an 11-day period. The primary outcome is pure-tone air-conduction thresholds measured 22 days after cessation of weapons training, including change from baseline as measured by absolute change and the frequency of STS. In addition, the study team is assessing tinnitus using questionnaires to assess changes in tinnitus loudness and annoyance.

D-Met has not been used in humans previously for the prevention of NIHL, although a variety of animal data support this indication. Translation to humans was facilitated by a Phase 1 safety study in which the pharmacokinetics of D-Met were assessed following oral administration of MRX-1024 (a formulation of D-Met) in normal volunteers (Hamstra et al. 2010). At that time, the patents protecting D-Met (Campbell 2001, 2008) were licensed by Molecular Therapeutics, Inc., which developed MRX-1024 for clinical studies. Testing in healthy volunteers was followed by Phase 1 testing in 25 patients receiving MRX-1024 concurrent with radiation therapy (RT) with or without weekly cisplatinum in an open-label study (Hamstra et al. 2010). Hamstra et al. (2010, p. 2666) report, "When administered concurrent with RT without chemotherapy, it was associated with a modest increase in grade 2 (two of six patients) and grade 3 (one of six patients) emesis. In those treated with MRX-1024 along with RT and weekly cisplatinum, there was no appreciable increase in emesis." After describing all study withdrawals due to nausea and emesis, the authors conclude, "Aside from nausea and emesis, there were no other significant toxicities observed related to MRX-1024." Development

later reverted back to Southern Illinois University School of Medicine. A new company, MetArmor, Inc., has recently been founded by Dr. Campbell to continue the clinical development of D-methionine as an otoprotective agent.

5.5.3 *Beta-Carotene, Vitamins C and E, and Magnesium*

Early data on prevention of NIHL using the combination of beta-carotene, vitamins C and E, and magnesium came from preclinical tests at the University of Michigan (Le Prell et al. 2007), and like the compounds described in Sects. 5.5.1 and 5.5.2, patent protection was sought before publication of preclinical data (Miller et al. 2011). This case study represents what can perhaps best be described as a hybrid development, as clinical trials were developed at academic institutions, funded by NIH, and completed with the help of industry patent licensees who formulated GMP supplies. This case study was initially introduced by Le Prell in Chap. 2, where the institutional review of NCT00808470 was described as encountering an "obstacle" in that University of Michigan leadership (the home of the parent NIH award NIH U01 DC008423) would not allow US studies evaluating the "nutraceutical" formulation to move forward without an IND. Under the requirements of an IND, the clinical trial materials were required to meet relevant pharmaceutical standards, including stability tests for all four active nutrient agents. Those tests are not currently required for dietary supplements marketed under the Dietary Supplement Health and Education Act of 1994 (DSHEA).

The initial licensee of these patents, OtoMedicine, Inc., undertook the expensive process of formulation, manufacturing, and purity and stability analyses. OtoMedicine, Inc., was dissolved before the collection of all FDA-required documentation and the manufactured supplies could not be used in the planned studies. This was a significant setback with respect to time and resources. A new licensee for the University of Michigan patents, Hearing Health Sciences, Inc., was identified, and they developed a chewable tablet formulation. An IND application was filed with CMC data in hand, and the study was allowed in the fall of 2012 under IND 116027. Although this was different from the originally proposed 2008 start date in the 4-year NIH grant that funded the conduct of these studies, the NIH allowed the period over which funding was awarded to be extended through multiple no-cost extension periods, so that the trial could begin enrollment once clinical materials and IND approval were in hand. Since then, specific guidance on studies on the prevention of acquired hearing loss has indicated that such studies *always* need an IND before use in a clinical trial (E. Bastings, The FDA/IND Process and Recommendations, presented August 24, 2012, Baltimore, MD); thus, FDA guidance should be sought regarding "nutraceutical" agents when planning clinical trials.

5.6 Summary

Some 16 % of disabling hearing loss worldwide has been attributed to occupational noise (Nelson et al. 2005). The burden is reduced in developed countries, with some 9 % of adult-onset hearing loss in the United States attributed to noise exposure (Nelson et al. 2005). According to NIOSH, "Four million workers go to work each day in damaging noise. Ten million people in the U.S. have a noise-related hearing loss. Twenty-two million workers are exposed to potentially damaging noise each year" (http://www.cdc.gov/niosh/topics/noise/stats.html). Industrial noise is not the only source of damage; concerts, nightclubs, and sporting events have the potential to be quite loud. Power tools and other devices can be hazardously loud as well. A key concern is the worker who receives 100 % of the maximum daily noise exposure allowed by OSHA, then goes home and engages in loud recreational activity or uses loud equipment or machinery at home (for discussion, see Le Prell 2016).

Ruben (2000) raises concerns that subtle, "preclinical" deficits have the potential for increasing impact on the modern workforce, even in the absence of an STS. Strong communication skills are critical to many in the workforce. Negative consequences of accumulating hearing impairment, such as difficulty hearing normal communication, communicating over the telephone, or understanding speech in a noisy office setting, are already reflected in labor statistics in the form of decreased income (Ruben 2000). Communication difficulties result in underemployment, or even unemployment, and as the American labor force increasingly shifts from manual labor to service-related occupations *that critically depend on communication abilities,* communication disorders will continue to emerge as an increasingly important public health issue for the 21st century (Ruben 2000).

Hearing conservation is achieved through engineering controls (decreasing noise at its source), administrative controls (decreasing the amount of time spent in a noisy environment), and personal hearing protection devices (HPDs: earplugs and earmuffs). Without doubt, HPDs will continue to be important tools for hearing loss prevention, and they continue to improve, with electronic technology representing a major step forward for preserving situational awareness (Casali et al. 2009; Talcott et al. 2012). Nonetheless, the hearing conservation amendment was promulgated more than 30 years ago (OSHA 1983), and NIHL remains one of the top disabilities for the workforce. There is active debate as to whether the regulations are adequate (Dobie and Clark 2014, 2015; Morata et al. 2015; Suter 2015). Even if the noise limits are deemed sufficiently protective, compliance with HPD requirements is frequently inadequate based on poor uptake of education, failure to correctly use earplugs, failure to consistently use HPDs, and so forth. Regardless of one's opinion on the "best" workplace noise limits, it is clear that additional strategies for protecting hearing are urgently needed, including quieter devices, better HPDs, and improved educational interventions. Ethical drugs that successfully navigate the review and approval process for the purpose of hearing loss prevention will provide an additional new tool for those at risk when noise is unexpected or earplugs are

unavailable or impractical. To reach this point, continued efforts to move scientific investigations from the laboratory into the clinical test environment are urgently needed.

Compliance with Ethics Requirements

Eric Lynch is a Co-Founder of and the Chief Scientific Officer for Sound Pharmaceuticals, Inc.

Jonathan Kil is a Co-Founder of and the Chief Medical Officer for Sound Pharmaceuticals, Inc.

Colleen Le Prell was Co-Principal Investigator on clinical study SPI-1005-202 with Sponsor Sound Pharmaceuticals, Inc. under IND 068443. She is a co-inventor on patents assigned to the University of Michigan and the University of Florida.

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