Citation: Clin. Transl. Sci. (2019) 12, 334–342; doi:

TUTORIAL

Regulatory Affairs 101: Introduction to Investigational New Drug Applications and Clinical Trial Applications

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Testing novel drugs on fellow human beings is fraught with potential ethical concerns; however, developing drugs to treat the wide spectrum of human diseases and disorders is a moral imperative. How do we best navigate the balance between protecting the individual vs. the greater good? Global government regulatory bodies are accountable for ensuring that medical experiments on human subjects are appropriately justified and subject to close oversight. In this article, we focus on two major global health authorities, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and the path to legally treating humans with new investigational products.

The US Food and Drug Administration and the European Medicines Agency

The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) are health authority bodies that regulate the use of investigational drugs within the United States and the European Union, respectively. In addition, investigational review boards (IRBs) in the United States and ethics committees (ECs) in the European Union must approve the use of drugs in humans; these entities are separate from health authorities, and their primary responsibility is ensuring the protection of human subjects who participate in studies testing new drugs.

The FDA is a part of the executive branch of the US government under the Department of Health and Human Services. As per their mission statement, "The Food and Drug Administration is responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices; and by ensuring the safety of our nation's food supply, cosmetics, and products that emit radiation."

The EMA was inaugurated in 1993 in an effort to ensure harmonization of the assessments and approval of drugs in Europe, and ensure fair access to innovative drugs to all European patients with no distinction of borders. Over the years, the European pharmaceuticals system has been evolving toward centralization under the EMA's umbrella, always with patient safety and access as highest priority.

Basis of ethical human medical experimentation

Global health authorities (HAs), including the FDA and the EMA, adhere to a set of principles called Good Clinical Practices (GCPs), which are the basis of modern human medical experiments and were developed to ensure the protection of human subjects participating in clinical trials. GCPs are rooted in, among other precedents, the Nuremberg Code (see **Box 1**: Nuremberg Code). The Nuremberg Code, which describes the tenets of ethical human research, was developed as a result of the Nuremberg trials of 1947.[1] During these trials, Nazi physicians claimed that horrific experiments conducted on prisoners were not illegal, as they adhered to common international research practice. In response, the Code outlines the conditions under which medical experimentation on human subjects is acceptable.

Today, GCPs are detailed in a guidance document, International Conference on Harmonization (ICH) E6(R2),[2] generated by a consortia of global HAs. In addition to guidance documents, such as ICH, detailed requirements governing all aspects of drug development, including manufacturing, nonclinical studies, clinical trials, safety monitoring, efficacy assessments, marketing, and postmarketing surveillance, are codified in the laws and regulations of global regulatory bodies.[3]

In this article, we discuss the information required by the FDA[4] and the EMA[5] to initiate and conduct medical experiments in human subjects. To enable the study of investigational drugs in human subjects, documentation must be submitted and reviewed by these HAs prior to administering an investigational drug to a human subject. In the United States, the initial submission to permit use of an investigational drug in a clinical setting is called an investigational new drug (IND) application. In the European Union, this documentation is submitted within a clinical trial application (CTA).

DATA REQUIRED TO SUPPORT INITIAL CLINICAL TRIALS

Administering a new drug to humans has inherent risk. Scientists, clinicians, and regulators strive to continuously improve how drug candidates are evaluated prior to the first-in-human (FIH) administration to minimize this risk as much as possible.

Years of research in multiple specialties are required to produce the totality of evidence necessary to support

Box 1 The Nuremberg Code

(1) The voluntary consent of the human subject is absolutely essential.

(2) The experiment should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study, and not random and unnecessary in nature.

(3) The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify the performance of the experiment.

(4) The experiment should be so conducted as to avoid all unnecessary physical and mental suffering and injury.

(5) No experiment should be conducted where there is an *a priori* reason to believe that death or disabling injury will occur; except, perhaps, in those experiments where the experimental physicians also serve as subjects.

(6) The degree of risk to be taken should never exceed that determined by the humanitarian importance of the problem to be solved by the experiment.

(7) Proper preparations should be made and adequate facilities provided to protect the experimental subject against even remote possibilities of injury, disability, or death.

(8) The experiment should be conducted only by scientifically qualified persons. The highest degree of skill and care should be required through all stages of the experiment of those who conduct or engage in the experiment.

(9) During the course of the experiment, the human subject should be at liberty to bring the experiment to an end if he has reached the physical or mental state where continuation of the experiment seems to him to be impossible.

(10) During the course of the experiment, the scientist in charge must be prepared to terminate the experiment at any stage, if he has probable cause to believe in the exercise of the good faith, superior skill, and careful judgment required of him, that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject.

advancing a new drug into human trials. The company sponsoring the development of a new drug (Sponsor) is required to provide a robust data set describing how the drug is made and determined to be pure and potent, the results of testing the effects of the drug in animals, and the plans for exploring exposure in humans as safely as possible. Here, we review the data required to support filing an IND in the United States[6,7] or a CTA in the European Union.[8,9]

Chemistry, manufacturing, and control information

Before a new drug can be administered to humans, the Sponsor must assure regulators that the drug is made under controlled conditions, with ongoing tests to ensure that the drug meets prospective criteria (e.g., identity, purity, potency, and stability). These data are required to show that a drug is what it is supposed to be, without any contaminants, and that it will maintain its purity and potency for at least the duration of the studies. Information on both the drug substance (the active pharmaceutical ingredient) and the drug product (the formulated drug ready for administration) must be submitted to the regulators. These data evolve over time as the Sponsor optimizes production and formulation of the drug.

Information on the drug substance should include the proper identification, quality, purity, and strength of the active ingredient, with an emphasis on the identification and control of raw materials and the new drug substance.

Information on the drug product is also provided, and similar to the drug substance section, this should include data supporting the assays and acceptable results for assessing its identity, strength, quality, and purity. It is also necessary to demonstrate stability (evidence on how the quality varies with time under the influence of a variety of environmental factors, such as temperature, humidity, light, etc.) for at least the duration of the clinical trial to inform the drug product shelf life and recommended storage conditions. Early in development, the drug product may be in a variety of forms; for example, it may be simply the drug substance handfilled into capsules, or it may be a biologic purified from a small-scale production.

Information on the placebo, if included in the clinical study, is also required.

Nonclinical pharmacokinetics, pharmacology, and toxicology information

A multitude of issues must be explored prior to dosing a human with a new drug. What happens biologically when a person takes a new drug? Does the drug stay in the body long enough to have any effect? Is it effectively delivered to the site of action? Is the drug metabolized, and what is the potential impact of the metabolites? Does the drug have an impact on how the body functions? If so, what body systems are impacted, and at what doses? Does the drug have any long-term positive or negative effects? Does the drug have potential to treat a disease or condition? If a drug has potential therapeutic benefit, do these benefits outweigh the potential risks?

Given the risks of administering a new drug to humans, a range of studies are conducted beforehand in test tubes, human and animal-derived cell lines, and animal models to explore the effects of the drug.

Distribution, metabolism, and pharmacokinetics. Pharmacokinetics refers to how a drug is processed through the body. These studies explore how the drug is absorbed once administered, where it distributes to in the body, how it is metabolized, and how it is excreted. These studies range from the identification of small molecule drug metabolites *in vitro* to the measurement of the drug in the blood, urine, and feces obtained from animals that are administered the drug.[10,11]

Box 2 Bial-Portela study[26,27]

In 2016, Bial-Portela was conducting a single-ascending dose (SAD) and multiple-ascending dose (MAD) study with BIA-102474-101, the 10th fatty acid amide hydroxylase to enter the clinic, as a potential treatment for pain. A total of 90 subjects completed treatment without incident in the SAD and first four MAD cohorts. In the fifth MAD cohort, one subject became ill after the fifth dose and was admitted to the hospital. Despite this, remaining subjects in the cohort continued to be dosed. Four of the five subjects who were dosed were eventually hospitalized, and the first subject incurred brain injury that resulted in death. BIA-102474-101 binds the enzyme fatty acid amide hydrolase (FAAH); however, it is a relatively unselective drug and, at higher exposures, it may bind other targets as well (known as "off-target binding"). Doses administered in the affected cohort of the BIA-102474-101 study were several-fold higher than required to fully inhibit FAAH. This tragedy led to a revision to the EMA's first-in-human (FIH) guidance, with revised recommendations for Sponsors, such as considering all FIH drugs as high risk; incorporating detailed stopping rules at the subject, cohort, and study levels; and better integration of the pharmacokinetic/pharmacodynamic data and modeling to determine the appropriate doses and schedule.[28]

Pharmacology. Pharmacology[12,13] is how a body responds to a drug and can be broken down into three categories: primary pharmacodynamics, secondary pharmacodynamics, and safety pharmacology.

Primary pharmacodynamics explores whether the drug has the intended effect *in vitro*: if it is a kinase inhibitor, does it inhibit the kinase in a test tube or cell line? If the drug is supposed to bind a cell-surface receptor, does it bind that receptor expressed on a human cell line? In other words, does the drug have the primary effect one would anticipate and at what concentration?

Secondary pharmacodynamics explores additional effects of the drug: does it bind any other proteins or antigens or receptors, and with what affinity? Does it inhibit any enzymes other than the intended target, and is the inhibition competitive or not? Secondary pharmacodynamics are key to assessing whether the drug has additional impact other than the intended effect, which may contribute to a better understanding of the overall safety profile of the drug. For example, this may include a dose-ranging study to assess drug binding with other human substrates *in vitro*. In the case of a kinase inhibitor, these studies would reveal what other enzymes to which the drug may bind and at what concentration. This information is critical to understanding the drug's possible "off-target" effects or the unintended side effects of drug treatment or overexposure (see **Box 2**: Bial-Portela Study).

Safety pharmacology experiments are designed to assess the impact of the drug on vital functions and are generally conducted in animal models, such as rodents and nonhuman primates. The core battery of these assessments includes the central nervous system, cardiovascular system, and respiratory system in animal species that are considered pharmacologically relevant. Selection of the pharmacologically relevant species is based on a number of factors, including whether the drug acts on the animal's system in a way that is similar to how it would act in humans (see **Box 3**: TeGenero Study for case study). The Sponsor may also conduct targeted safety pharmacology studies in other systems if there may be a known or suspected impact.

Toxicology. Toxicology[14–16] studies are conducted primarily in animal models and are designed to predict, as much as possible, any potential toxicities (i.e., adverse or negative outcomes) in humans who may be exposed to the drug. The extent of these studies varies by indication: drugs designed to treat life-threatening diseases may not require the same level of evidence to support FIH studies as drugs designed to treat a more chronic disorder. For example, prior to initiating clinical trials, most drugs are evaluated for the risk of genotoxicity (when the drug changes the recipient's DNA such that a mutation could be passed to their offspring) using a bacteria-based assay, whereas oncology drugs are not required to evaluate genotoxicity until prior to marketing.

Generally, toxicology studies are required for all drugs to explore the adverse effects of the drug when administered to one or more animal models as a single dose and after multiple doses. These studies attempt to predict the highest dose that could potentially be administered to humans before encountering toxicities. Once toxicities are identified

Box 3 TeGenero study[29,30]

In 2006, TeGenero AG initiated a phase I study in healthy human volunteers with a single intravenous dose of their anti-CD28 antibody, TGN1412. TGN1412 was a candidate for the treatment of B-cell lymphomas and autoimmune disorders based on its potential to expand the T-cell population in the absence of T-cell receptor activation. TGN1412 had been assessed extensively in nonclinical studies; importantly, these studies did not appropriately predict the human immune response. The starting dose in humans was 500× below the no-observed-adverse-effect level (NOAEL). Six healthy volunteers received TGN1412 doses within minutes of each other. Within 90 minutes, the subjects began to feel ill and, within 24 hours, all 6 were hospitalized with cytokine-release syndrome that resulted in organ failure. All subjects survived, although they may have long-term disability, including one subject who required amputation of fingers and toes. The analysis of this tragedy informed the European Medicines Agency's subsequent guidance on first-in-human trials.[28,31] The guidance included recommendations for Sponsors to consider using the minimal anticipated biological effect level rather than NOAEL in determining the human starting dose, staggering drug administration in subjects; ensuring that dosed subjects are observed for adverse events prior to initiating dosing in subsequent subjects; including additional risk mitigations for drugs that are considered to be "high risk"; and ensuring that phase I studies are conducted by qualified investigators with access to emergency medical services.

in animals, these studies assess whether or not the toxicities are reversible. For oncology drugs, data from studies of 28 days in two different animal species support continuous dosing in patients, as long as the patient is deriving benefit. Toxicology studies with a duration similar to that in the planned clinical trial are required for nononcology drugs. Results from these studies are critical in evaluating whether the potential benefit outweighs the potential risk of administering the drug to humans.

Additional toxicology studies, including the drug's risk of causing cancer or reproductive harm, may be evaluated later in the lifecycle of the drug development.

Clinical protocol

The clinical protocol is the step-by-step detailed plan of exactly how the new drug is to be evaluated in humans. The critical chemistry, manufacturing, and control (CMC) and nonclinical safety assessment data collected to date are briefly summarized and then integrated into the study plan. For example, the chemistry of a small molecule drug will determine whether the drug can be formulated into an orally available pill or if it will be administered intravenously. Predictions from pharmacokinetic studies will drive how often the drug is administered, and the nonclinical safety findings in animal models from the toxicology studies will inform the drug dose and how human subjects are monitored for potential adverse side effects.

Key aspects of the protocol include the following:

- Study population: In designing the clinical trial in which a new drug is administered to humans for the first time (an FIH study), Sponsors decide whether it is safe to test the drug in healthy volunteers or whether it would be more appropriate to test the drug in the intended patient population, where the acceptable risk tolerance may be higher. For chronic diseases, it is critical that the clinical safety profile supports long-term administration. It may be preferable to initially evaluate these drugs in healthy volunteers, where the safety assessment is not complicated by the underlying disease. In contrast, many oncology drugs have significant adverse effects, and may only be initially tested in patients with late-stage cancer, in whom the risk/benefit profile of the drug is acceptable. Another aspect to assess is whether the drug may have negative long-term consequences in the treated population. For example, in the 1990s, a study was conducted in patients with renal failure who were administered recombinant human erythropoietin to treat anemia. Some of these patients generated antibodies against their endogenous erythropoietin in response to treatment with the recombinant erythropoietin, leading to an autoimmune disorder called pure red cell aplasia.[17]
- Dose selection: Based on the nonclinical safety assessments, a starting dose that is unlikely to result in adverse side effects is selected (see **Table 1**). A common FIH study design is to test the drug at a low dose in a small number of subjects and assess for safety concerns for a duration determined by the drug's pharmacokinetic and pharmacodynamic profile. If the safety is acceptable, a higher dose level is similarly assessed in another small group of subjects. This dose-escalation paradigm is continued until an appropriate pharmacodynamic dose level is safety attained, unacceptable safety findings are noted, or the dose level approaches an exposure at which unacceptable safety findings were observed in nonclinical studies. In some studies, additional subjects are assessed at this dose level to obtain further safety data. In some patient populations, such as rare diseases, in which there are only a small number of patients in which to assess the drug's safety and efficacy, it may be important to select a dose that may have the potential for direct benefit for patients enrolled in an FIH study.[18]
- Safety monitoring plan: A clinical safety monitoring plan is designed to assess subjects for any toxicity that may have been identified by the nonclinical studies, may be of particular importance to the patient population, or may be a class effect of drugs that hit the same target or pathway. The Sponsor will list which potential adverse events may arise, at what exposures, and at what duration after the drug is administered. Based on this, subjects will undergo safety assessments during the conduct of the clinical study.

Table 1 Determining a safe starting dose (FDA and EMA Guidance)

Step 1: Determine the NOAEL
NOAEL is the highest dose tested in a nonclinical study that does not produce a significant increase in adverse effects compared to the control group.
Alternatively, Sponsors may use MABEL, which is typically more conservative than NOAEL, as it is based on any pharmacological activity and not toxicity and may be used for drugs with higher risk.
Step 2: Calculate the HED
HED is calculated from the exposure defining the NOAEL in each nonclinical toxicology study most commonly by normalizing to body surface area or body weight.
Alternatives include scaling based on drug levels if dose is limited by local toxicity (e.g., topical drugs) or based on volume if administration is limited by compartment (e.g., intrathecal administration).
Step 3: What is the most appropriate species?
Sponsors will have several HEDs based on various nonclinical studies. The most appropriate HED is selected based on the most appropriate pharmacologically relevant species.
Interestingly, the most sensitive species is not always the most relevant (e.g., low doses of NSAIDs causes gastrointestinal lesions in beagles but are well tolerated in humans).[32]
Step 4: The safety factor
Nonclinical toxicology studies may not perfectly predict the human adverse event profile. To account for differences between nonclinical models and humans, Sponsors apply a safety factor, typically 10-fold, to the HED to get the recommended starting dose.
This safety factor may be increased for higher risk drugs (e.g., if there is a nonmonitorable toxicity or nonlinear pharmacokinetics). Alternatively, this factor may be reduced for a drug that belongs to a well-characterized class or if toxicities are monitorable, predictable, and reversible.
Step 5: Consider the estimated PAD
Sponsors should consider the estimated PAD to determine a starting dose. The FDA suggests that the PAD may be a more sensitive estimate of possible toxicities and may warrant lowering the starting dose to below the PAD. The EMA recommends that the starting dose is below the PAD in healthy volunteer studies.

EMA, European Medicines Agency; FDA, US Food and Drug Administration; HED, human equivalent dose; MABEL, minimal anticipate biological effect level; NOAEL, investigational new drug; NSAIDs, nonsteroidal anti-inflammatory drugs; PAD, pharmacologically active dose.
Guidance refs. 9,33.