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Safe Starting Dose and Dose-Escalation Schemas in First-In-Human Studies with New Molecular Entities

**Dissertação no âmbito do Mestrado em Biotecnologia Farmacêutica,
orientada pelo Professor Doutor Sérgio Paulo de Magalhães Simões
e pelo Professor Doutor José Luís de Almeida
e apresentada à Faculdade de Farmácia da Universidade de Coimbra.**

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"Ninguém é perfeito

Eu sou um suspeito

Desta poesia que é minha"

António Poeiras Valério

ABSTRACT

There is an increased need for the development of new, effective and safe drugs due to new diseases, related to the increase in average life expectancy, and due to the lack of better treatments for already existing illnesses. The approval of new molecular entities is only possible through the conduction of nonclinical and clinical studies, where animal and human data is evaluated, respectively.

First-in-human studies are a key step in the drug development process, since they represent the first time in which the new drug is administered to humans. Such studies have non-therapeutic objectives and involve the assessment of safety and tolerability as well as the determination of the safe dose range for following studies. However, they constitute a major challenge in drug development because not much is known about the behavior of new drugs in humans, regarding their safety, toxicity, pharmacodynamics and pharmacokinetics.

Safety in first-in-human studies is ensured by the correct determination of the safe starting dose and by the definition of dose-escalation schemas. Since humans face an element of risk and in order to prioritize their safety and well-being, all data must be acknowledged in order to put in place mitigation measures prior to the first exposure to the new drug. Moreover, recent incidents, that led to the harm of several subjects, promoted the development and refinement of strategies to protect their safety, while ensuring scientific progress.

Thus, it is of utmost value to correctly conduct first-in-human studies, not only to protect all subjects, but also to ensure the creation of reliable data for further studies, and, consequently, lead to the approval of innovative drugs.

Keywords: First-In-Human Studies; New Molecular Entities; Safe Starting Dose; Dose-Escalation Schemas; TGN1412.

RESUMO

Existe uma necessidade crescente de desenvolver novos medicamentos eficazes e seguros devido a novas doenças, relacionadas com o aumento da esperança média de vida, e devido à falta de melhores tratamentos para doenças já existentes. A aprovação de novas entidades moleculares só é possível através da condução de ensaios não-clínicos e clínicos, onde informação de animais e seres humanos é avaliada, respetivamente.

Ensaios de entrada no homem são um passo essencial no processo de desenvolvimento de fármacos, uma vez que estes ensaios representam a primeira vez em que o novo medicamento é administrado em seres humanos. Estes estudos têm objetivos não terapêuticos e envolvem a avaliação de segurança e tolerabilidade, bem como da determinação do intervalo de dose segura para estudos seguintes. No entanto, constituem um grande desafio no desenvolvimento de fármacos, pois pouco se sabe sobre o comportamento de novos medicamentos em seres humanos, no que diz respeito à sua segurança, toxicidade, farmacodinâmica e farmacocinética.

A segurança em estudos de entrada no homem é garantida pela correta determinação da dose segura inicial e pela definição de esquemas de aumento de dose. Uma vez que os seres humanos enfrentam um elemento de risco e se pretende priorizar a sua segurança e bem-estar, toda a informação não-clínica deve ser reconhecida, de forma a colocar em prática todas as medidas de mitigação antes da primeira exposição ao novo medicamento. Para além disso, incidentes mais recentes, que causaram danos à saúde de vários participantes, promoveram o desenvolvimento e o apuramento de estratégias para proteger a sua segurança, ao mesmo tempo que o progresso científico é assegurado.

Portanto, é de extrema importância conduzir corretamente estudos de entrada no homem para proteger todos os participantes, mas também para garantir a criação de informação de confiança para estudos seguintes e, consequentemente, para levar à aprovação de medicamentos inovadores.

Palavras-chave: Ensaios de Entrada no Homem; Novas Entidades Moleculares; Dose Segura Inicial; Esquemas de Aumento de Dose; TGN1412.

ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, and Excretion
AUC	Area Under the Curve
BLA	Biologics Licence Application
BSA	Body Surface Area
CDER	Center for Drug Evaluation and Research
CBER	Center for Biologics Evaluation and Research
CEIC	Comissão de Ética para a Investigação Clínica
CHMP	Committee for Medicinal Products for Human Use
CL	Clearance
CNPD	Comissão Nacional de Proteção de Dados
CNS	Central Nervous System
CV	Cardiovascular
DLT	Dose-Limiting Toxicity
ECG	Electrocardiogram
EMA	European Medicines Agency
F	Bioavailability
FDA	Food and Drug Administration
FIH	First-In-Human
GLP	Good Laboratory Practices
HED	Human Equivalent Dose
HNSTD	Highest Non-Severely Toxic Dose
IB	Investigator's Brochure
ICH	International Conference on Harmonization

IND	Investigational New Drug Application
IRB	Institutional Review Board
INFARMED	Autoridade Nacional do Medicamento e Produtos de Saúde
MABEL	Minimum anticipated biological effect level
MOA	Mechanism of Action
MRSD	Maximum Recommended Starting Dose
MTD	Maximum Tolerated Dose
NME	New Molecular Entity
NDA	New Drug Application
NOAEL	No Observed Adverse Effect Level
PD	Pharmacodynamics
PK	Pharmacokinetics
RNEC	Registo Nacional de Estudos Clínicos
RO	Receptor Occupancy
RP2D	Recommended Phase II Dose
STD10	Severely Toxic Dose in 10% of rodents
U.S.	United States
WOCBP	Women of Childbearing Potential

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I – INTRODUCTION TO FIRST-IN-HUMAN STUDIES

1.1 First-in-Human Studies

A long time as passed since the first clinical study was conducted, when James Lind tested the idea that citric acids could cure scurvy (a disease that took the life of many sailors in the 1700's). Since then a lot has changed, but not before several atrocious medical experiments were made, such as the ones performed during World War II. However, the Nuremberg Code, in 1947, and the Declaration of Helsinki, in 1964, came to improve the conduction of clinical research by establishing the rights of human subjects. Currently, clinical research is under strict regulation and is conducted in order to prioritize the safety and well-being of every subject.[1]

A new drug must undergo nonclinical and clinical studies before being ready to be marketed. Nonclinical studies usually occur before the clinical part of the development; however, they can be conducted in parallel, and are comprised of animal, in vitro and/or ex vivo experiments. Information retrieved from nonclinical studies is used to plan subsequent studies in humans. Clinical studies are divided in four different phases, from I to IV, each one with individual goals. Phase I, II and III must be concluded before the introduction of new drugs in the market, whereas phase IV begins after commercialization. [2]

First-in-Human (FIH) studies belong to phase I and are one of the most important studies, since they represent the first contact between the new drug and humans. FIH studies can generate the first information about new therapies from humans and, subsequently, help design further studies.[3] The collection of data regarding safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) are the primary objectives of FIH studies. These studies are non-therapeutic, since they do not involve the assessment of therapeutic value. [4] In regard to safety and tolerability, these studies are used to estimate the tolerability of different doses and to detect adverse reactions. In what concerns PK, the absorption, distribution, metabolism and excretion (ADME) of a new drug is accessed, in order to determine how the drug is managed by the system and to gather information about the possibility of accumulation. Later, it will be important to study special populations, such as elderly, children and subjects with hepatic or renal impairments, which can originate different PK information. As regards to PD, it can provide the necessary data to understand how drugs affect the human organism, serve as a guide for dose selection in following studies and offer an initial evaluation of efficacy.[2]

The evaluation of food effects on drugs and biomarker assessment can contribute to further characterize new drugs during this phase. Food-drug interactions can have an impact on safety and efficacy. For that reason, it is important to determinate if, and to what extent, food impacts exposure of the drug and if the effect is different for different food contents.[5] It was discovered that biomarkers, defined as any characteristic that is measured as indicator of health, disease or a response to an intervention, play a crucial role to enhance the efficiency and speed of drug development. Clinical endpoints measure how a patient feels, functions, or survives; however, clinical outcomes can take much time to study. Therefore, the use of surrogate endpoints, defined as biomarkers that are expected to predict the effect of a therapeutic intervention and usually measured in serum or plasma, allow the conduction of clinical studies with less subjects in shorter periods of time. Since FIH studies represent the first opportunity to assess biomarkers in humans, it is critical to implement biomarker programs in this phase. [6]

It is demanded that all FIH studies should be conducted in specific clinical research units equipped with standardized procedures, adequately trained staff and appropriate medical and emergency care. An evaluation is done before the beginning of the study in order to ensure that all facilities are qualified and that all site staff is trained and capable to work with new drugs. [4]

Switching from animals to humans will always be one of the biggest challenges when it comes to clinical research. Since FIH studies are the first studies to be conducted in humans, they constitute a challenge in drug development, since little is known about the new product, having only nonclinical data to support their conduction. The complexity of these studies must be recognized in order to plan an appropriate study that involves several key aspects to protect all subjects and achieve success in following studies, such as, the correct safe starting dose and dose-escalation schemas. [4]

1.2 Key Stakeholders and Main Responsibilities in FIH Studies

There are several participants involved in the conduction of clinical studies, called stakeholders:

- **Subjects:** Individuals, either healthy or patients, that participate in a clinical study.[7]

- **Sponsor:** A person or organization responsible for the conception, conduction, management or financing of a clinical study.[7]
- **Monitor:** An individual designated by the sponsor to supervise the progress of a clinical study by ensuring that is conducted according to the protocol, standard operating procedures, good clinical practices and applicable regulatory requirements. [8]
- **Principal Investigator**: Leader of the team responsible for the conduction of a clinical study in a study site. [9]
- **Contract Research Organization**: Company hired by the sponsor to perform regulatory activities, monitoring, data management or even pharmacovigilance activities throughout the duration of clinical studies. [9]
- **Regulatory Authorities:** Authorities responsible for the review and approval of all documentation associated to the submission of clinical studies. In Portugal, this responsibility belongs to the *Autoridade Nacional do medicamento e Produtos de Saúde, I.P* (INFARMED), *Comissão de Ética para a Investigação Clínica* (CEIC) and the *Comissão Nacional de Proteção de dados* (CNPD). [8]
- **Study site:** Location where clinical studies are conducted with adequate materials and staff. [8]

When considering the planning of a FIH study, other stakeholders can be recognized:[10]

- **Formulation Scientist:** Individual that researches, develops and optimizes product formulations.
- **Safety Physician:** Person who evaluates drug performance and elaborates safety monitoring plans.
- **Clinical Development Scientist**: Person responsible for providing scientific support for product development, such as, input on study design.
- **Regulatory Affair Specialist**: Person who contributes for the attainment and management of drug approvals, through regulatory consultation and submission of clinical studies.
- **Toxicologist**: Individual who assesses the potential risks associated with new drugs and provides assistance in the development of nonclinical studies.
- **Clinical Operations Specialist**: Assists in study site selection, approvals and CRO collaborations. Ensures the correct conduction and quality of clinical studies.
- **Clinical Pharmacologist**: Person who supports the elaboration of study protocols, by ensuring all nonclinical findings are considered in the development of the study.

1.3 Study Population

In order to conduct a proper FIH study, the selection of the population to include must be addressed. Investigators need to consider all scientific, ethical and safety requirements in order to elaborate eligibility criteria. These criteria play an important role, since they target specific population and once defined serve as guidance to recruit appropriate participants. However, if too restrictive they can become an obstacle to implement to real world subjects. In this context, selection of subjects must be carefully considered to protect the safety of all subjects and to guarantee the quality of data obtained. Subjects can either be healthy or patient: [4]

- Healthy subjects, which by definition, from the World Health Organization, means that these subjects are in "a state of complete physical, mental and social well-being and not only in the absence of disease or infirmity". [11]
- Patient, when subjects suffer from the disease that is intended to be treated with the new drug.

The decision to include healthy or patient subjects should be made on a case-by-case basis and account for the safety of subjects and the quality of data to be created. Most of FIH studies are performed with healthy subjects. Nevertheless, whenever a high-risk agent is involved, such as cytotoxic drugs targeting life threatening conditions, patients are enrolled, because the risk-benefit assessment would be unfavorable for healthy subjects.[10] Furthermore, the presence of comorbidities and concomitant medication jeopardize results and consequent interpretation. As a result, healthy subjects younger than 60 years old are included in FIH studies in order to exclude those factors. There is an increase in the number of studies recruiting patients and certain study designs may need to use both subjects, switching from healthy subjects to patients. Several PROs and CONs regarding the selection of either healthy subjects or patients are depicted in Table 1. [4]

Table 1: Selection of Healthy Subjects and Patients [4]

	Healthy subjects	Patients
PROs	-Easier and quicker management and recruitment -No concomitant comorbidities or medications -Data may be useful for several indications -High internal validity	-PD/biomarker data may only be obtainable in patients -Target-related safety may be tested -Possible benefit, especially at higher doses -High external validity
CONs	-Often no or limited target-related PD/biomarker data -Higher difficulty to justify target availability -Target-related safety may be different from patients -PK may be different from patients -No therapeutic benefit to subjects -Low external validity	-Recruitment and management are more difficult -Concomitant disorders and medications make interpretation difficult -Target-related safety may still be different in other indications -Single or low doses may not provide adequate therapeutic benefit to justify the inclusion of very ill patients -Ethical concerns around placebo use

Special populations must be discussed, due to their unique risk/benefit considerations.[2]

a) Pregnant Women

The inclusion of pregnant women in clinical studies presents a challenge, since there is a risk of harming the well-being and development of the fetus or embryo, particularly in FIH studies where the risk is even higher. The inclusion of pregnant women in clinical studies is justified after adequate reproductive and development toxicology studies in animals are completed and in case there is data regarding the use during pregnancy. Usually, phase I and phase II must be completed before the enrollment of pregnant women. [12]

b) Women of Childbearing Potential (WOCBP)

There is a level of concern about including WOCBP, because of unplanned exposure of an embryo or fetus. For this reason, some precautions may be implemented before taking part in a FIH study to minimize the risk of exposure: [4]

- Conduction of nonclinical reproduction toxicity studies, in order to identify the risk of the new drug on reproduction and development;
- Access pregnancy status by making pregnancy tests before enrollment and during the conduction of the study;
- Consent to use highly effective birth control during the course of the study to prevent pregnancy;
- Warning about the potential risks of pregnancy during the duration of the study.

In case WOCBP get pregnant, they should be discontinued from the study and pregnancy must be followed until its completion. [2] Furthermore, If WOCBP are included before the completion of reproduction toxicity studies, participation must be of short duration (less than 2 weeks) and essential to meet the objectives of the clinical study. [13]

c) Geriatric

Geriatric population is defined as 65 years or older. Special attention is required, since the presence of age-related diseases and concomitant drug can lead to adverse reactions and drug interactions. The enrollment of geriatric subjects is, mainly, in phase III clinical studies. [14]

d) Pediatric

FIH studies are usually conducted in adult healthy subjects and new drugs should only be tested in healthy children if all guidelines are followed. All studies will be conducted in this population except for initial safety and tolerability data that will be obtained from adult healthy subjects, for drugs intended to be used exclusively by children. In cases where data obtained from adults is not useful, new drugs may only be studied in this population (e.g. surfactant for respiratory distress syndrome and therapies for metabolic or genetic diseases unique to children). For serious or life-threatening diseases, studies with children should begin after a safety and potential benefit evaluation. [15]

Study subjects, usually, receive a monetary compensation for their participation in phase I clinical studies. In order to protect the interests of subjects, all risks and benefits

involved in their enrollment as well as compensation details must be clarified in the informed consent.[4]

1.4 Sample Size and Inclusion of Placebo Subjects

A correct determination of the sample size limits unnecessary exposure to hazardous treatments, while ensuring proper results. In addition, if neglected it may cause the approval of an ineffective drug or the exclusion of an effective drug. [16]

The estimation of sample size will depend on power, type I and type II errors. Power, in statistical terms, it is the probability of rejecting the null hypothesis when it is indeed false. In FIH studies, power is very low, due to the small sample size; only events with high occurrence are detectable with adequate power. Type I error occurs when a true null hypothesis is rejected, which is the probability of attributing an event to a drug when it is not due to that drug. Type II error results from accepting a false null hypothesis, the probability of not identifying an event when the drug causes that event. Such information combined with a series of events caused by the new drug is used to develop a curve that shows the detectable event rate as a function of active cohort size and power (Figure 1). [10][16]

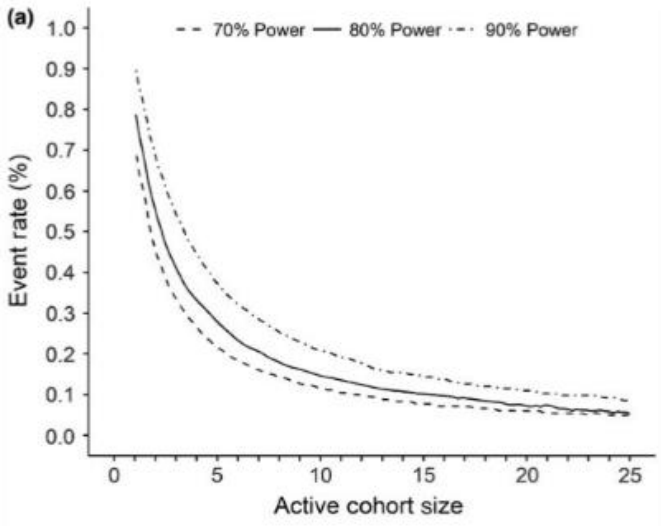

Figure 1 Detectable Event Rate as a Function of Active Cohort Size and Power [10]

Increasing the cohort size, in cohorts with less than 6 subjects, results in a decrease in the detectable event rate, and when increasing the cohort size above 10, results in a flattening of the curve, indicating that there is not much to gain with an increase of subjects (Figure 1).

For this reason, sample size between 6 and 10 subjects is acceptable. Additionally, background rate (rate of occurrence of events not related to the drug) must be taken into consideration, since increasing sample size results in the observation of more events not related to the drug. Sample size needs to be large enough to be able to make inferences about, however it cannot be too large, since many subjects will be exposed to an unproven treatment and background rate will increase. If sample size, on the other hand, is too small it is more likely to generate inconclusive or incorrect results. [10] [17]

Another aspect that dictates sample size is the inclusion of placebo subjects. Placebo has been used as control group in clinical research and FIH studies usually include these subjects to make a distinction about safety measures, such as adverse events, and PD endpoints between active and placebo subjects. However, many specialists consider the inclusion of placebo subjects in FIH studies useless. In order to ensure the blind, further documentation and procedures are required, followed by increased costs and time. Additionally, decision to escalate doses is based on observed adverse events and in this case placebo subjects will negatively influence the conduction of the study. Therefore, the added value of placebo subjects in FIH studies must be evaluated and its inclusion must be justified. [18]

1.5 Safety Monitoring

Safety monitoring is an essential part of all stages of the drug development cycle and as a result, sponsors must create safety monitoring programs. However, safety monitoring presents a challenge in FIH studies. The detection of safety signals, defined as data on adverse events that needs additional investigation, can be difficult due to the small size of FIH studies. [19] Additionally, studies conducted in patients increase the complexity of safety monitoring because comorbidities and disease progression can contribute to an incorrect detection of adverse events.

Therefore, in order to effectively detect safety signals, adequate data collection and analysis combined with the comprehension of the limitations of study designs are required. Data collection comprises a combination of several safety assessments such as physical exams, vital signs, clinical laboratory tests and electrocardiograms (ECGs). Other assessments must be required depending on results from nonclinical toxicity studies. The enrollment of an independent monitoring committee is not always necessary; however, they are useful to determinate if a dose is unsafe. [10]

Ultimately, the design of a safety monitoring plan must be complete before the initiation of an FIH study, in order to define which parameters must be analyzed and scheduling measurements. [10]

1.6 Regulation of Clinical Studies

Regulation of clinical studies is carried out by the European Medicines Agency (EMA) or the United States Food and Drug Administration (FDA), depending if an approval is expected in the European Union or in the United States (U.S.). The main purpose of these organizations is to ensure the safety and wellbeing of all subjects and efficacy of the new product, while granting a rapid regulatory process to commercialization.

i. European Union (Portugal)

In Portugal, the conduction of clinical studies for human use is regulated by the *Lei n.º 21/2014, de 16 de Abril*. This law covers the regime for conducting clinical studies with drugs for human use which transposes into the national law the Directive 2001/20/CE of the European Parliament and of the Council of 4 April 2001, regarding the application of good clinical practices in the conduction of clinical studies for all member states. [8]

The conduction of clinical studies requires the submission of a clinical study application through an electronic platform, the *Registo Nacional de Estudos Clínicos* (RNEC), to the following entities by the sponsor: [8]

- **INFARMED, I.P**: The clinical study application must contain the following data: the protocol; Investigator's Brochure (IB); full identification of the sponsor and principal investigator; identification and qualification of all members of the investigation team; identification of all sites; identification of all regulatory authorities and experimental medicine dossier. This entity has the responsibility to authorize the conduction of clinical studies within a maximum of 30 days. During this period, INFARMED, I.P can request complementary information or documents.
- **CEIC**: The independent organism that grants the protection of rights, safety and wellbeing of all participants in clinical studies must issue a favorable opinion within 30 days and can also request complementary information and documents.
- **CNPD:** The commission that authorizes the processing of health data carried out in the context of the clinical study must decide within 30 days.

Complementing this legislation, a set of guidelines that cover all areas related to clinical studies are available at Volume X of Eudralex, known as the collection of rules and regulations regarding medical products directed at clinical studies in the European Union. [20]

INFARMED, I.P issued a statistical analysis in order to understand the evolution of clinical studies applications over the years. Between 2006 and 2011 there was a sharp decrease of submitted clinical studies. However, after 2012 the number of clinical studies register a positive evolution. Phase III clinical studies continue to have the greatest expression in Portugal, followed by phase II. Nevertheless, it is important to mention that applications for phase I clinical studies have been increasing over the years (Figure 2). [21]

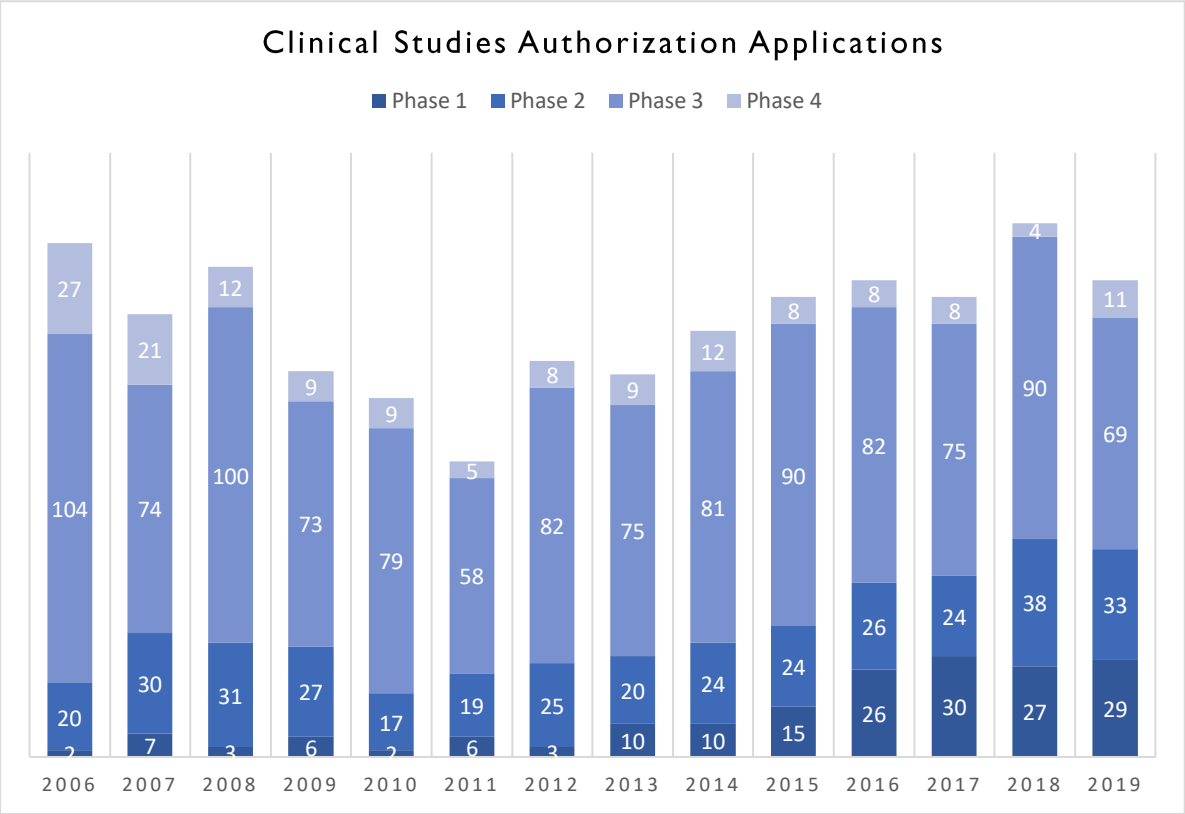

Figure 2 Clinical Studies Authorization Applications Per Phase of Clinical Development [21]

ii. United States

The submission of an investigational new drug (IND) application to the FDA is required before conducting clinical studies. Part 312 of the Code of Federal Regulations covers the IND that must be complete for unapproved drugs and for already approved drugs for new indications. These applications should include data from nonclinical studies, manufacturing information and the clinical protocol. FDA can either allow the conduction of clinical studies

or put the IND on clinical hold, which means that a clinical investigation should be delayed. Response by the FDA should be given within 30 days. [22]

Pre-IND consultation program is recommended before submitting an IND. These meetings intend to promote early conversations between sponsors and the FDA in order to receive feedback and discuss the drug development program. They can be very important to reduce time and costs. [22]

The protocol can be simultaneously submitted to institutional review boards (IRB) in order to obtain approval. IRB is an independent committee equivalent to CEIC, that intends to protect the safety and wellbeing of all subjects. Only after the protocol is approved by the IRB and the IND is approved by the FDA, recruitment and enrollment can start.

FDA releases every year the number of IND applications. Data shows that submissions reached a steady state between 2014 and 2016, however, in recent years have been increasing with 2018 being the best year so far (Figure 3). [23]

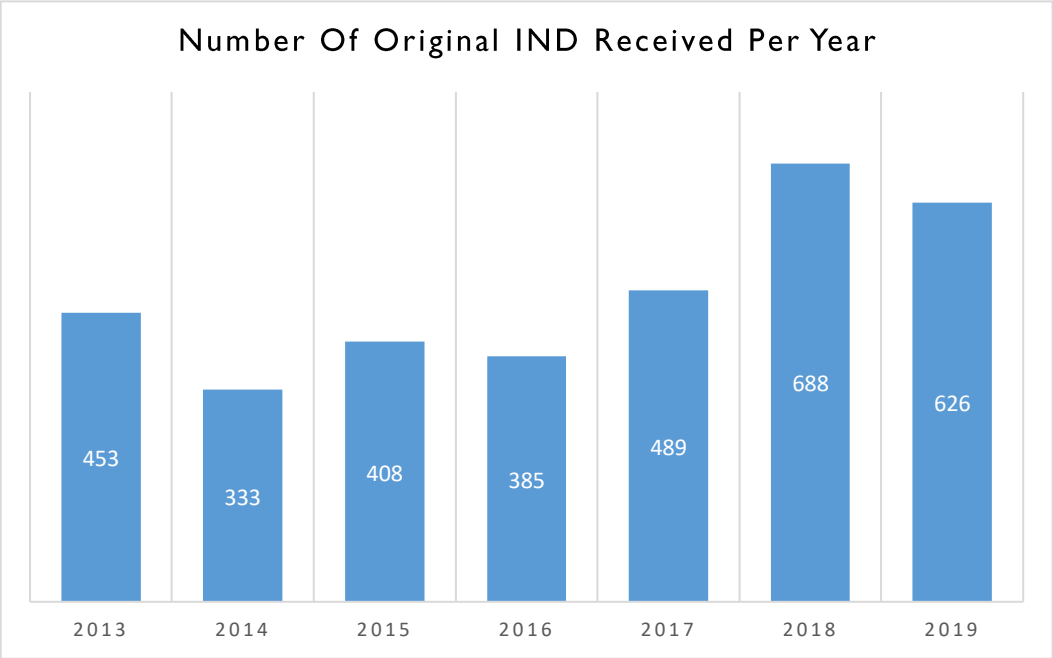

Figure 3 Number of Original IND Received Per Year [23]

1.7 Clinical Studies Success Rates

The onset of new diseases, due to the increased global average life expectancy associated with an aging population, results in an increase demand for new, effective and safe drugs. For that reason, sponsors are making efforts to decrease the technical uncertainty of a new drug before reaching later and more expensive stages of development, such as phase II or III.

A new study carried out at Massachusetts Institute of Technology estimated clinical studies success rates by using a sample of 406 038 entries of clinical study data since 2000 until 2015. After an extensive analysis, researchers concluded that the overall success rate continued to decrease until 2013. However, since 2013 an increase has been observed, reversing the paradigm. Regarding the distinct phases of clinical studies, phase I and phase II followed the same path as success rates continued to decrease until 2013. Phase III, on the other hand, remained stagnant and in 2013 a substantial increase was observed. (Figure 4). The increase after 2013 can be explained by the fact that many sponsors are now better at identifying potential failures. This way, only new drugs that have a higher probability of success move on to the next stages, which leads to higher productivity. Furthermore, recent use of biomarkers allows compounds to be targeted at patients who may have a better response. Lastly, several medications are being development for different indications which leads to higher success rates. [24] Phase I has high success rates since phase transition is not dependent on efficacy, unlike phase II that shows lower success rates because it is the first time that efficacy of the new drug is tested. Also, after phase II sponsors usually terminate clinical research, in order to avoid moving to larger and more expensive studies, such as phase III.

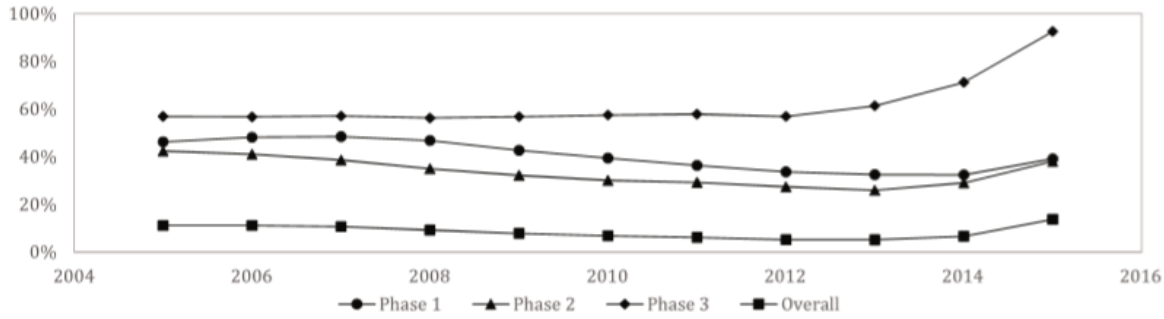

Figure 4 Probability of Success Over the Period of January 1, 2005 and October 31, 2015. [24]

II – NEW MOLECULAR ENTITIES

2.1 Drug Discovery Process

The pharmaceutical industry is making efforts to keep up with the increasing demand for innovative drugs, however the introduction of drugs in the market is complex and time-consuming. The process starts with the discovery of a new compound that can take, in average, from three to six years to be complete. The drug discovery process is composed of several stages: Target Identification, Target Validation, Lead Compound Identification and Lead Compound Optimization. [25]

a) **Target Identification**

During target identification, *in vitro* research is conducted to identify targets involved in certain diseases. Targets must have promising properties in order to ensure the success of drug development; therefore, targets must be efficacious, safe, not evenly distributed across the body and address clinical needs. Additionally, to be a good target, it must be "druggable", meaning that it must be accessible, and its activity can be modulated by a drug compound. Proteins and genes are some examples of biological targets. [25]

b) Target Validation

After identifying a target, validation intends to demonstrate that the target is involved in the progression of a disease and it can be regulated. This step leads to a higher confidence in the relation between targets and diseases and if conducted carefully results in an increased probability of success. [25]

c) **Lead Compound Identification and Validation**

This step consists of the identification or development of a compound that can interact with the selected target. Investigators either evaluate natural compounds that can be later turned into drugs or, as an alternative, design synthetic drugs that can interact with the target without cross-reactivity. Safety testing is initiated during this phase as well as PK and PD studies, providing initial information about metabolism and physiological effects.[25]

d) Lead Compound Optimization

Optimization of the previously discovered lead compound is essential. The main goal is to preserve desired properties and reduce defects. Chemical structures may be modified to

improve target specificity and selectivity, avoiding off-target interactions. Ideal dosage and route of administration are tested during this phase. [25]

2.2 Definition of New Molecular Entities

I. European Union

The term new molecular entity (NME) is generally not used in Europe. Medicinal products or new active substances are applied instead. According to the Directive 2001/83/EC of the European Parliament, medicinal product is defined as any substance or combination of substances (with either human, animal, vegetable or chemical origin) that: [26]

- are intended to treat or prevent diseases in humans;
- are applied to restore, correct or modify physiological functions;
- implement a pharmacological, immunological or metabolic action.

New active substances comprise new molecules from both chemical and biological sources, defined as: [27]

- Substances not previously authorized as a medicinal product;
- Derivate of a previously authorized chemical substance but with distinct safety and efficacy;
- Biological substance already authorized with different molecular structure, source of nature or manufacturing method;
- Radiopharmaceutical substances that are radionuclides; the ligand or the linking mechanism has not been authorized.

II. United States

In the U.S., new drugs are classified as NME that can either be: [28]

- Small molecule drugs approved by Type 1 new drug application (NDA). NDAs are classified in ten different types. Type 1 refers to drugs that contain an active ingredient that has an active moiety (molecule or ion responsible for the pharmaceutical properties of the drug) not yet approved by the FDA under section 505 of the Act or marketed as a drug in the U.S. Type II to type X are referred as non-NME;
- New biological drugs approved by Biologics License Application (BLA).

There are several types of drug products that can become available in the market, such as:[4]

- **Chemical entities:** chemical substance used to treat diseases;
- **Cell therapy products:** restore or alter cells;
- **Gene therapy products:** replace, inactivate or introduce genes;
- **Plasma-derived products:** derived from human plasma, such as albumin;
- **Immunological products:** such as vaccines;
- **Herbal products**: derived from plants;
- **Homeopathic products:** natural alternatives medicines;
- **Radiopharmaceutical products:** contain radioisotopes.

Four different groups can be created in order to differentiate drug products: [26] [29]

- **Orphan drugs:** Group of drugs used to treat rare diseases.
- **New non orphan drugs:** Group of new drugs used to treat all conditions except for rare diseases.
- **Biosimilars:** Group of drugs highly similar to biological drugs that present no significant differences from the reference product.
- **Generics:** Group of drugs identical to already existing drugs in terms of composition of active substances with demonstrated bioequivalence.

2.3 Marketing Authorization Procedures

Once all information is gathered about the new drug, specially, when a drug is believed to be effective, submission to regulatory agencies is initiated. Such agencies will evaluate efficacy, safety and determine if the benefits outweigh the risks. In certain cases, a higher level of risk may be acceptable, for example, in treatment of terminal diseases. Certain drugs may be considered as more urgent than others and, for that reason, are prioritized in the approval process. These drugs are given priority if they provide treatment that did not exist until that point. [29]

i. European Union

Market introduction is only possible after competent authorities issue a marketing authorization that consists of a decision that grants authorization and a dossier with relevant

data according to Articles 8(3) to 11, of Directive 2001/83/EC and Annex I and with Articles 6(2) of Regulation (EC) No 726/2004 and Article 7 of Regulation (EC) No 1394/2007. In the European Union, there are four procedures to market a new drug based on the product and in the number of countries intended to be marketed: [29]

a) Centralized Procedure

Marketing authorization is granted by the European Commission, after consulting the Committee for Medicinal Products for Human Use (CHMP). CHMP makes an extensive scientific evaluation of data in order to determine if new drugs meet all requirements of quality, safety, efficacy and a positive risk-benefit balance. This marketing authorization is valid in all member states. [29]

When a new drug is of major public health interest, sponsors can request for an accelerated process with clear justification. Orphan medicinal products, intended to treat rare diseases, fall under the centralized procedure. Several regulations aim to encourage the development of new drugs by providing incentives, such as, a 10-year period of market exclusivity. In addition, biosimilar medicinal products are required to be authorized by the centralized procedure. Advanced therapies, new medicinal drugs that use gene therapy, cell therapy and tissue engineering also fall under this procedure. [29]

b) **National Procedure**

National procedures are used for drugs intended to be marketed in only one-member state. Sponsors must submit an application to competent authorities of that member state. [29]

c) **Mutual Recognition Procedure**

Under this procedure member states can rely on scientific assessments made by other member states. A medicinal product must already have been approved in one-member state. The marketing authorization holder must submit an application using this procedure to receive a marketing authorization in a concerned member state. The country designated to evaluate the application is called the reference member state. [29]

d) **Decentralized Procedure**

This procedure refers to products that do not fall under the centralized procedure, request approval in more than one-member state and that have not been marketed in any EU state. A reference member state will evaluate the application. [29]

INFARMED, I.P is responsible for marketing authorization in Portugal with its specific procedures and its recognized as a reference member state with active participation in mutual recognition and decentralized procedures.

ii. United States

Marketing authorization can be achieved by NDA or BLA application in the U.S. Documentation should contain all data throughout every step of the drug development process, such as, results from animal and clinical studies, how it is manufactured, processed and packaged. The Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) are responsible for approvals. There are three NDA pathways under Section 505 of FD&C Act and one BLA pathway under Section 351 of the Public Health Service Act: [30]

a) **505(b)(1) NDA**

The 505(b)(1) regulatory pathway is used to get approval of a new drug whose active ingredients have not been previously approved. An extensive research is required in terms of safety and efficacy from nonclinical and clinical studies conducted by the sponsor. For this reason, this type of submission may take several years to be complete. [31]

b) **505(b)(2) NDA**

This pathway was conceived to avoid duplication of studies of already approved drugs, since safety and effectiveness data from previous studies can be used to meet requirements. For this reason, sponsors may be provided with shorter approvals. Some potential candidates include drugs with new indications, changes in dose or different route of administration. [31]

c) **505(j) Abbreviated New Drug Application (ANDA)**

505(j) ANDA is used to market an identical version of a previously approved drug. Generic drugs do not require animal or clinical studies, instead bioequivalence studies must be performed in terms of active ingredients, dosage form, route of administration, strength, conditions of use and labeling. Some differences are allowed only if further investigation is not necessary to establish safety and effectiveness. [31][32]

d) BLA

BLA consists of a request to introduce a biological drug into commerce. Data retrieved from animal and clinical studies must establish safety, purity and potency of the product. Since the production of biological products is complex, inspections before the submission are common. Biologics can either be approved by CDER or CBER. [30]

2.4 Marketing Authorization Decisions

EMA and the FDA provide documents that reflect the continuous trend of bringing to the market the most diversified and innovative drugs. The following information was retrieved after an extended research in both EMA and FDA websites regarding the approvals of new non orphan drugs, orphan drugs, biologics and generics.

i. EMA

In 2019, CHMP issued 66 positive opinions, of which 41 were related to new medicinal products. It is visible that over the years the number of new non orphan drugs has been decreasing. In fact, the number of positive opinions reached a valley in 2016. For orphan drugs an increase has been observed in the last decade, as well as, for biosimilars; these approvals reached the highest peak in 2018 with 21 approved orphan drugs and 15 approved biosimilars. Generics do not follow a uniform path, but it is expected that they will continue to belong to one of the most approved drug groups. The year 2018 turned out to be a good year, since approvals started to increase after the disappointment observed in 2016 (Figure 5). [33]

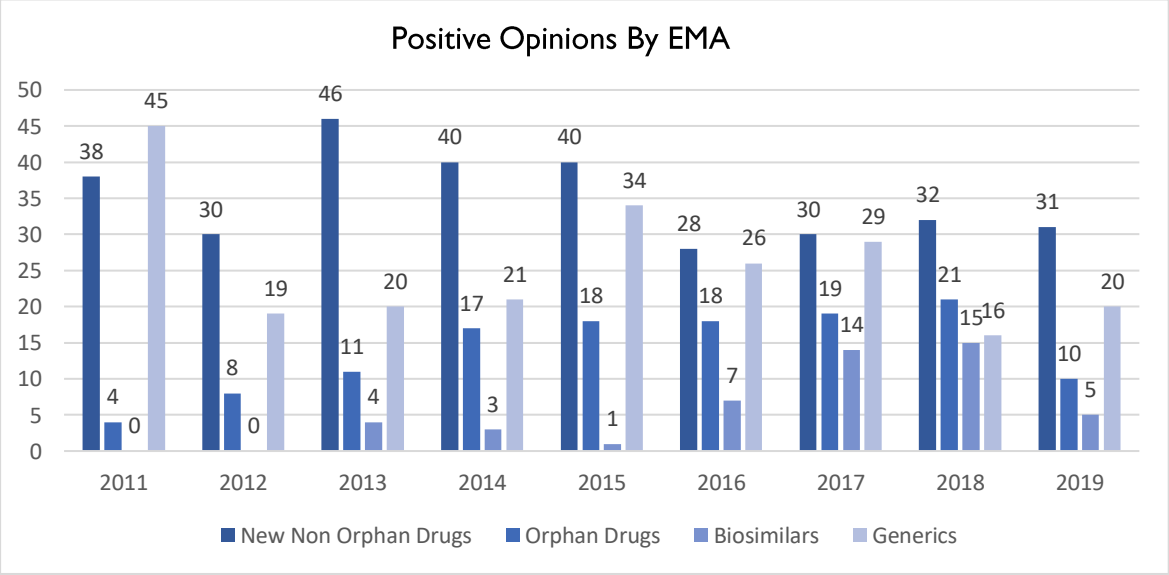

Figure 5 Positive Opinions by EMA [33]

ii. FDA

Novel drug approvals by CDER and CBER have been increasing throughout the years, except for 2016, where a lowest point was reached (Figure 6). This can be due to the fact that several drugs had been expected to be approved in 2016, however, ended up being approved in 2015, which allowed drugs to be available sooner. Additionally, in this year, several applications failed because of lack of compliance with good manufacturing practices. [34] Approvals for orphan and biosimilars drugs have been increasing. Regarding generics, they represent the group that has the most approvals, but they seem to follow the same tendency as the other groups.

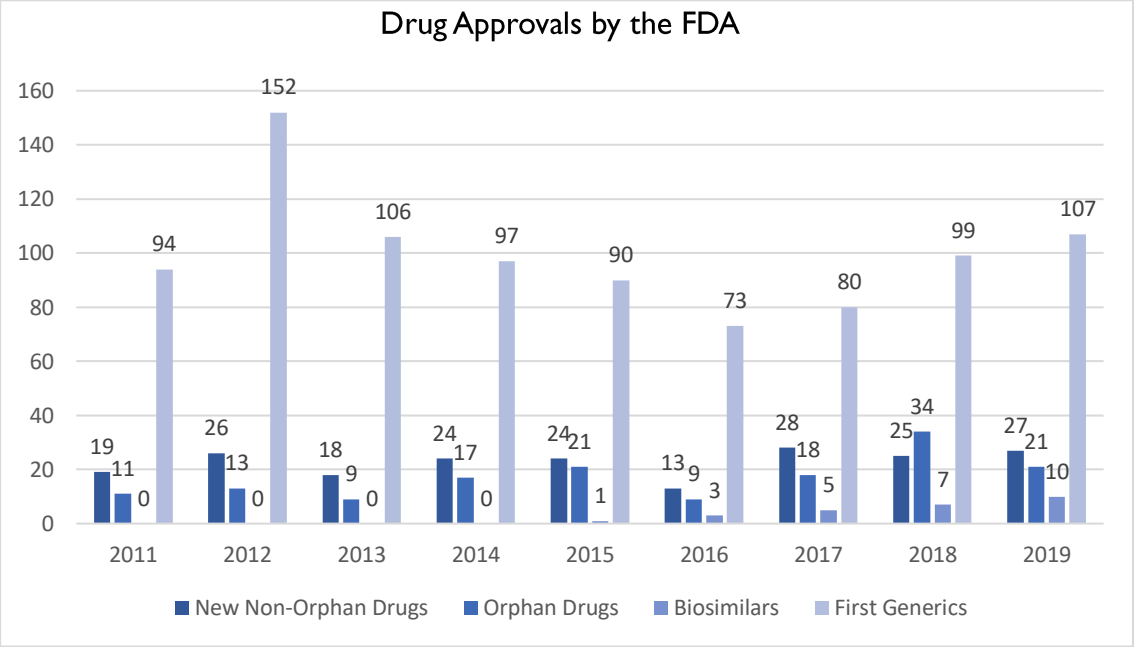

Figure 6 Drug Approvals by the FDA [34]

iii. EMA and FDA Comparison

A year to year comparison between EMA and the FDA shows that approvals are largely in tune; peak or valley years affect both agencies in the same way. Actually, more than 90% of authorizations between these two regulatory agencies are aligned, as a consequence of increased dialogue and cooperation since 2003. However, disparities about efficacy and clinical data to support an application are quite common between both agencies. [35]

The year 2018 turned out to be exceptional for both agencies. Rare-disease therapies are continuously increasing over the years, with the help of incentives created to support the development of such drugs.

Generic drug approval by the FDA is significantly larger than EMA, which can be due to the Generic Drug User Fee Act, GDUFA, created by the FDA to speed the approval process of new generic drugs. [36]

Both in the European Union and in the U.S. the time it takes for regulatory agencies to give their approval is crucial to define how long it will take to market a new drug. It is known that FDA reviewing times are shorter, leading to faster and, consequently, more drug approvals. [37]

2.5 New Molecular Entities Success Rate in Clinical Studies

In 2016, a new study of clinical development success rates, from 2006 to 2015, was carried out in the U.S. A total of 9.985 phase transitions, when a drug advances to the next phase of development or is suspended, were analyzed. [38]

Data shows that non-biologic NMEs have the lowest transition success rates over all phases of development while non-NME, were proven to be the ones with the highest success rates in every phase of development. Non-biologic NMEs have the lowest probability of success (61%) when transitioning from phase I to phase II and with only 6.2% of likelihood of approval, which is the probability of being FDA approved, while biologics have almost twice the likelihood of approval (Figure 7). [38]

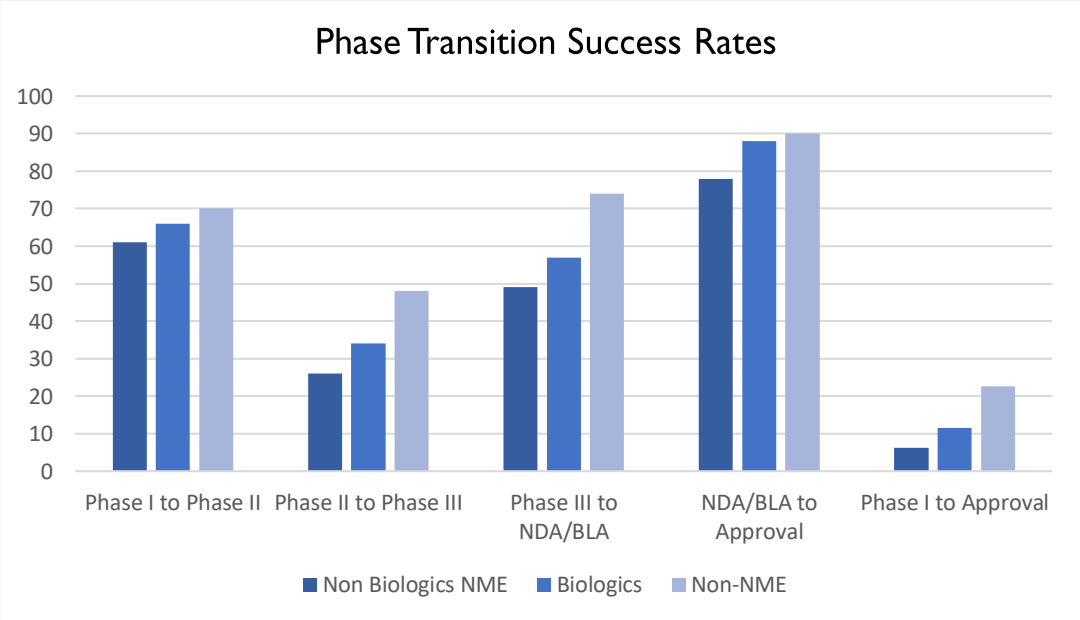

Figure 7 Phase Transitions Success Rates [38]

Non-NME have a three-time higher likelihood of approval when compared to non-biologic NMEs. These results are not surprising, since non-NME include reformulations or combinations of already approved products. [38]

Biologics are associated with higher success rates in comparison with non-biologic NMEs as a result of the lack of off-target toxicity, usually referred as the main cause of failure in small molecules. In addition, biologics undergo further validation processes during nonclinical studies, which allows them to move towards clinical studies with greater confidence. [38]

Success rates may have also been influenced by several other aspects. Clinical validation of a target is an essential part of the drug discovery process, and the better the evaluation, the better are the expected results. Additionally, a higher extent of knowledge of the mechanism of action (MOA) prevents errors during clinical research, but its novelty can potentially lead to uncertainty, and consequently, failure. Patient population and selection criteria are extremely important and when carefully chosen are believed to produce higher confidence in results. Finally, it is not uncommon for drug development programs to be terminated due to lack of funding or due to the complexity of the clinical study, further impacting overall success rates. [38]

III – SAFE STARTING DOSE

3.1 Nonclinical Safety Testing

Clinical studies and marketing authorization of a new drug are supported by the conduction of nonclinical safety studies. Information retrieved from nonclinical safety testing is critical to estimate the safe starting dose and dose range for clinical studies. In addition, such data will enable the detection of biomarkers for safety monitoring. Common objectives of these studies include the identification of organ toxicity, dose dependence, relationship to drug exposure and potential reversibility. [13] In order to achieve such goals, the type of drug (small molecules or biologics), therapeutic indication, the scope and design of the FIH study will dictate which studies must be conducted and if additional assessments are required. [10]

Several efforts have been made in order to achieve harmonization with the creation of the International Conference on Harmonization (ICH), that brings together regulatory agencies and industry to discuss and respond in a consistent way to the rapid increase of drug development. Such project was initiated by Europe, Japan and the U.S., in 1990, and consisted of the creation of guidelines for the development and registration of new therapies. [39] Given the fact that not all pharmaceuticals are identical, several guidelines that recommend safety evaluation programs were created. The ICH provides the ICH M3(R2) for pharmaceuticals, the ICH S6(R1) for biotechnology-derived pharmaceuticals and the ICH S9 for anticancer drugs. [10]

The quality and integrity of nonclinical studies are secured by the conduction of studies in compliance with Good Laboratory Practices (GLP), a quality system that controls how nonclinical studies are performed. Nonclinical safety testing comprises the following studies: pharmacology, acute toxicity, repeated dose toxicity, toxicokinetic and pharmacokinetic, reproduction toxicity, genotoxicity and carcinogenic potential. Further studies can be conducted on a case by case basis, such as phototoxicity, immunotoxicity and abuse liability studies. [13]

a) Pharmacology Studies

Pharmacology studies consist of primary pharmacodynamic, secondary pharmacodynamic and safety pharmacology studies. Primary pharmacodynamic studies are focused on the MOA and the effects caused by a new drug on the therapeutic target and can aid in dose selection for clinical studies. Whereas secondary pharmacodynamic studies are

intended to discover potential off-targets effects and their MOA. PD studies should be conducted *in vitro* and *in vivo* and overall do not need to be conducted in compliance with GLP. [40]

Safety pharmacology studies identify and evaluate potential undesirable PD effects of a new drug on vital organ systems: a) cardiovascular (CV) system by measurements of blood pressure, heart rate and an ECG; b) respiratory system, where respiratory rate and hemoglobin oxygen saturation are evaluated and c) central nervous systems (CNS), that will assess motor activity, behavioral changes and coordination. These studies must follow GLP and can be conducted *in vitro,* to establish a concentration-effect relationship and *in vivo*, to define dose-response relationship of the observed effect. Follow-up studies may be necessary when concerns for human safety arise. [40]

In addition, there is a need to assess the potential of a new drug to delay ventricular repolarization and prolong QT interval, which are linked to ventricular tachyarrhythmia. [41]

b) Toxicokinetic and Pharmacokinetic Studies

Toxicokinetics studies aim to characterize systemic exposure and its connection to dose level and duration of toxicity studies through the generation of PK data. PK parameters can be derived from several measurements (e.g. plasma concentrations) during toxicokinetics studies. Serum levels, area under the curve (AUC), maximum concentration (C_{max}) and maximum concentration at a specified time (C_{time}) are the most used to assess exposure in these studies. It is important to integrate PK parameters and information about ADME of a new drug into toxicity studies (e.g. single and repeated-dose toxicity studies) to help interpret and clarify toxicity findings.[42] Further studies that determine distribution and accumulation of the new drug are required as well as *in vitro* metabolic and plasma protein binding data. [43]

c) Single Dose (Acute) Toxicity Studies

During single dose toxicity studies, the new drug is administered in one or more doses for no longer than 24h. Two mammalian species are used, one rodent and one nonrodent. These studies can be useful to determine doses for repeated dose toxicity studies and to identify target organs of toxicity. [13]

d) Repeated Dose Toxicity Studies

Repeated dose toxicity studies are conducted to determine the toxicity of the new drug after repeated administration and identify exposure-response relationships. During the conduction of these studies two mammalian species are used, one rodent and one nonrodent. The duration of these studies should be the same or higher than the duration of human clinical studies (Table 2). [13]

Table 2 Recommended Duration of Repeated Dose Toxicity Studies to Support the Conduct of Clinical Studies [13]

Maximum Duration of Clinical Study	Recommended Minimum Duration of Repeated-Dose Toxicity Studies	
	Rodents	**Non-Rodents**
Up to 2 weeks	2 weeks	2 weeks
Between 2 weeks and 6 months	Same as clinical study	Same as clinical study
>6 months	6 months	9 months

Biotechnology-derived pharmaceuticals require a recovery period in order to determinate worsening and delayed toxic effects. The length of repeated dose toxicity studies depends on the intended duration of clinical exposure and disease indication: [44]

- For most biotechnology-derived pharmaceuticals: 1-3 months.
- Short-term use (≤ 7 days) and acute life-threatening diseases: up to 2 weeks.
- Chronic indications: 6 months with clear scientific justification.

e) Immunotoxicity Studies

Immunotoxicity consists of the suppression or enhancement of the immune response, which can lead to a reduced ability to fight infectious agents and to the development of autoimmune diseases, respectively. [45]

All new drugs should be tested with immunotoxicity studies and the following data usually indicates immunosuppression or enhancement of the immune response: hematological changes, changes in immune system organ weights and histology, increased occurrence of infections and tumors. Additional studies may be required when there is any cause for concern.

The nature of the immunological changes determine the type of additional studies to be conducted. [45]

Most of biotechnology-derived pharmaceuticals are designed to enhance or suppress the immune system. For that reason, routine studies are not usually used for these drugs.[44]

f) Genotoxicity Studies

Genotoxicity studies are used to detect genetic damage and usually conducted *in vitro* and *in vivo*. Ames test evaluates the capacity of a new compound to induce mutations in the DNA of bacteria and is proven to be extremely effective in the detection of relevant genetic changes. Chromosomal damage can also be evaluated *in vitro* and/or *in vivo*.[46] All genotoxicity tests must be completed before phase II clinical studies and must be in compliance with GLP. In case the compound can cause damage, an evaluation and extra testing are required. [13]

Regarding anticancer pharmaceuticals, genotoxicity studies are not required to support clinical studies for treatment of patients with advanced cancer. [47] The same is applicable for biologics, since these products will not interact with DNA. [44]

g) Carcinogenicity Studies

Carcinogenicity studies aim to detect carcinogenic potential for drugs intended to be used for at least 6 months or frequently in an intermittent matter. On the other hand, drugs used sporadically or in short duration, do not require these studies, unless there is cause for concern, such as previous demonstration of carcinogenic potential. [48]

Standard carcinogenic studies for biotechnology-derived pharmaceuticals are generally not required, however, they might be necessary based on the duration of clinical dosing, population and activity of the drug. Furthermore, genotoxic compounds must be submitted to these studies since they are assumed to be trans-species carcinogens. [44] [48]

h) Reproduction Toxicity Studies

Reproduction toxicity studies intend to detect any effect on reproduction caused by the new drug. Such studies are usually conducted in rats and aim to detect effects on: a) fertility and early embryonic development; b) pre- and postnatal development, including maternal function and c) embryo-fetal development, that are conducted in two species, one rodent and one nonrodent and are not required for patients with advanced cancer. [49]

Men can be included in phase I and II studies without male fertility studies; however, these studies must be completed before phase III clinical studies. For women with no childbearing potential, such studies are not necessary if repeated-dose toxicity studies were performed; although for WOCBP, the conduction of reproductive toxicity studies is required. [13]

i) Photosafety Testing

In vitro and *in vivo* photosafety testing, is necessary to avoid toxicity in humans. Photosafety testing is connected to effects such as phototoxicity and photoallergy, described as a tissue response and an immunologically mediated reaction to a chemical, respectively. Tests should be completed before phase III and whenever phototoxicity studies demonstrate potential risk the inclusion of a warning on the informed consent and in product information is essential. [13] [50]

j) Nonclinical Abuse Liability

Studies that are conducted in order to evaluate if a new drug has abuse potential can be conducted *in vitro* and *in vivo* and do not need to meet the requirements of GLP. Drugs that have abuse potential are CNS active and can generate mood changes and hallucinations. Drugs that present similarities to other drugs with no abuse potential, do no warrant these studies, however drugs with new MOA require abuse liability studies. [51]

3.2 Safe Starting Dose Selection

The most important step before proceeding with FIH studies is the determination of the safe starting dose, based on data retrieved from extensive nonclinical studies, that, consequently, is adjusted for humans. Even though this action is extremely common in clinical research, there is still limited uniformity or standardization of approaches. Since there is no agreement regarding the best method, it may be influenced by training and expertise of sponsors and investigators. [52]

Estimating a safe starting dose demands for a collaboration between scientists to determine which method is the most appropriate considering the properties of the drug. In order to ensure the safety and wellbeing of all subjects the ideal starting dose must be safe and close to a dose with minimal PD effects. In cases where the starting dose is too high it can

lead to the development of severe adverse effects and, on the other hand, if it is too low it can extend the dose-escalation process, causing the delay of clinical studies and the use of a greater number of subjects. [52]

There are several approaches that can be used to calculate the starting dose: NOAEL approach, PKPD Modeling, MABEL Approach, PK Modeling, Similar Dose Approach and STD10 and HNSTD Approach.

3.2.1 NOAEL Approach

This approach is intended to determine the Maximum Recommended Starting Dose (MRSD) for systemically administered small or large drugs. It is not applicable for endogenous hormones and proteins and prophylactic vaccines. This method is based on the following steps: [53]

1. Determination of the No Observed Adverse Effect Level (NOAEL).
2. Conversion of the NOAEL to Human Equivalent Dose (HED).
3. Selection of the most appropriate species.
4. Application of the safety factor.

1. Determination of the NOAEL

According to the FDA, NOAEL is the highest dose that does not cause a substantial increase in adverse events when compared to the control group. The NOAEL is considered as a reference of safety for safe starting dose determination, usually expressed in mg/kg. NOAEL must not be confused with: No Observed Effect Level (NOEL) that is related to any effect; Lowest Observed Adverse Effect Level (LOAEL), the lowest dose that produces an adverse effect or Maximum Tolerated Dose (MTD) that corresponds to the highest dose that will achieve a therapeutic effect without unacceptable adverse events. [53]

The NOAEL is identified after the assessment of all data from toxicity studies in animals, particularly about overt toxicity, surrogate markers of toxicity and excessive PD effects. Generally, NOAEL should be based on effects observed in toxicity studies that would be undesirable if originated in FIH studies. [53]

2. Conversion of the NOAEL to HED

Allometric scaling is an empirical approach that converts animal dose into human dose based on normalization of dose to body surface area (BSA), by using existing data in other species. [54]

The NOAEL is converted to HED based on BSA correction factor ($W^{0.67}$) that depends on the animal weight, with appropriate conversion factors from animal species; this method applies an allometric exponent (b) of 0.67, because it leads to a more conservative safe starting dose. The conversion factors convert the mg/kg dose in animals in mg/kg dose in humans, which corresponds to the NOAEL on a mg/m^2 basis. Standard conversion factors are shown in column F and G of table 3, however, for species and for weights not listed, conversion factors can be calculated with formula 1: [53]

Formula 1: Conversion factor = $(W_{animal}/W_{human})^{1-b}$

Thus, HED can be calculated depending if species and weights are present or absent from table 3. If this data is absent from table 3, HED can be calculated based on: [53]

Formula 2: HED (mg/kg) = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{(1-0.67)}$

For species and weights present in table 3, two other equations are presented in order to calculate HED: [54]

Formula 3: HED (mg/kg) = Animal dose (mg/kg) : conversion factor (column E in table 3)

Or

Formula 4: HED (mg/kg) = Animal Dose (mg/kg) x conversion factor (column G in table 3)

Table 3 Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area [53]

A	B	C	D	E	F	G
Species	Reference Body Weight (kg)	Working Weight Range (kg)	Body Surface Area (m^2)	Convert Animal Dose in mg/kg to Dose in mg/m^2 **Multiply by K_m**	Convert Animal Dose in mg/kg to HED in mg/kg. Either: **Divide Animal Dose by:**	**Multiply Animal Dose by:**
Human	60	--	1.62	37	--	--
Mouse	0.020	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.080	0.047-0.157	0.016	5	7.4	0.135
Rat	0.150	0.080-0.270	0.025	6	6.2	0.162
Ferret	0.300	0.160-0.540	0.043	7	5.3	0.189
Guinea Pig	0.400	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.9-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Monkeys	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.350	0.140-0.720	0.06	6	6.2	0.162
Squirrel Monkey	0.600	0.290-0.970	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro-pig	20	10-33	0.74	27	1.4	0.730
Mini-pig	40	25-64	1.14	35	1.1	0.946

Another conversion factor, K_m, can be used in order to convert a mg/kg dose into mg/m^2 (column E), which depends on the actual weight and surface area of test species: [53]

Formula 5: $Mg/m^2 = K_m \times mg/kg$

Conversion based on BSA is not recommended in case of drugs administered by topical, intranasal, subcutaneous and intramuscular routes or administered into divisions that have lower distribution outside of that division. In addition, proteins administered intravascularly with M_r (relative molecular mass) > 100.000 daltons should not be based on this approach. [53] A different dose normalization may be used in these cases, such as equating the HED to NOAEL [HED (mg/kg) = NOAEL (mg/kg)] with clear justification. This type of conversion is also used when: [53]

- NOAELs take place at a similar mg/kg dose through species;
- In case only two NOAELs can be obtained in different species and one of the following statements is true:
    - The drug is intended to be administered orally;
    - Toxicity in humans is dependent on an exposure parameter (eg. C_{max}) or other pharmacologic and toxicologic endpoints (eg. MTD) that are correlated across species on a mg/kg basis;
    - Strong relationship between plasma drug levels and dose in mg/kg.

3. Most appropriate species selection

The selection of the most appropriate species is the next step following the calculation of the HED. Most appropriate species are defined, as a default, as the most sensitive, which are the ones that originate the lowest HED. However, if another species, other than the most sensitive, is proven to be better at assessing human risk, the HED of that species should be used. The choice of the most appropriate species is influenced by differences in ADME of the drug between species and previous studies that prove that a specific animal model is more appropriate in the evaluation of safety. For certain biologics, other factors should be taken into consideration, such as whether that species expresses specific receptors or epitopes. [53]

4. Application of the safety factor

After the selection of the HED from the most appropriate species, a safety factor is applied, in order to further ensure the safety of the first dose. This safety factor allows that several results from nonclinical studies that raise concerns can be accommodated, such as:

differences between humans and animals regarding sensitivity to pharmacologic activity, receptor quantity, affinity and ADME; unexpected toxicities and challenges in toxicity identification. [53]

In order to obtain the MRSD, HED is usually divided by 10, which is the default safety factor. Although, this value should be based on an evaluation of the available data, the novelty of the drug, PD aspects, relevance of animal models and uncertainties related to the dose calculation, which can lead to its increase or decrease. Increasing the safety factor may be necessary when there is an increased concern about safety and, on the other hand, it may be lowered when available data ensures safety. The following information should be considered when choosing to adapt the safety factor: [53]

- Increasing the safety factor

The safety factor should be increased when any of the following concerns is identified: steep dose/response curve; severe toxicities; variable bioavailability; irreversible toxicity; unexplained mortality; large variability in doses or plasma drug levels eliciting effect; nonlinear PK; inadequate dose-response data; new targets and animal models with limited utility. [53]

- Decreasing the safety factor

This approach can be applied when drugs within the same class are administered by the same route, schedule and duration as well as when metabolic profile, bioavailability and toxicity are similar. Other aspects such as toxicities that are easily monitored, reversible, predictable and that display a stable dose-response relationship in all tested species can lead to the decrease of the safety factor. [53]

This approach can be complemented with the comparison of the MRSD obtained from the NOAEL with the Pharmacologically Active Dose (PAD), which refers to the lowest dose that has the target pharmacologic activity. Although HED is usually derived from NOAEL, if the PAD is obtained from *in vivo*, HED can be calculated from it with the use of a conversion factor. In case that the HED calculated from PAD is lower than the MRSD it may be necessary to decrease the safe starting dose. [53]

3.2.2 PKPD Modeling

PKPD modeling represents an alternative approach in order to obtain the safe starting dose for FIH studies. This modeling is a combination of a PK model, that describes the concentration-time course in body fluids derived from the administration of a drug dose, and a PD model, which describes the relationship of the concentration and the resulting effects. Thus, PKPD modelling links these two parameters to characterize the time course of the effects resulting from a dose regimen. The PKPD modeling consists of the following steps: [55][56][57]

1. Description of non-human concentration-response relationship.
2. Interspecies differences and human concentration-response relationship.
3. Prediction of human PK parameters.
4. Combination of PK and PD data.

1. Description of non-human concentration-response relationship

This step consists of the establishment of concentration-response relationship from species in nonclinical studies. As a result, PD models are developed leading to the determination of affinity, potency and efficacy of the new drug; EC_{50} is a common measure to establish the potency of a new drug and corresponds to the required concentration to induce a 50% effect. Such properties are used to identify the drug candidate with the best characteristics. This phase is also used to identify biomarkers. [56][57]

2. Interspecies differences and human concentration-response relationship

Interspecies differences must be addressed during this step, in order to identify variations in tissue distribution, receptor occupancy (RO) (quantifies the binding of a drug to its target on the cell surface and is usually a biomarker for this method), tissue and plasma protein binding and blood cell binding. If any significant differences in target expression are found between species, affinity and EC_{50} are expected to be different. Biological drugs are known to have higher interspecies differences in drug affinity. [56][57]

Concentration-response from nonclinical studies must be adapted to predict human concentration-response relationship and account for interspecies differences. In cases where there is only one animal model, *in vitro* studies that used animal and human receptors can contribute to establish differences between species. If there is no data to determine

interspecies differences, the effect of a certain concentration of a drug must be recognized as the same in the least sensitive species. [56][57]

3. Prediction of human PK parameters

After the human PD model is established, human PK parameters, such as clearance (CL), bioavailability (F) and concentration-time profiles must be extrapolated from nonclinical data. CL refers to the rate at which a drug is eliminated, while F refers to the percentage of a drug that reaches systemic circulation. Concentration-time profiles are useful since they can describe the area under the curve (AUC) that corresponds to the overall drug exposure, C_{max}, C_{min} (minimum drug's concentration in blood system) and $t_{1/2}$ (half-life) that corresponds to the time at which a drug reaches half of its value. Since these parameters have been proven to be useful in the prediction of FIH dose they must be scaled from animals to humans. Interspecies scaling can be made by allometric scaling or by physiological-based pharmacokinetic models (PBPK). The PBPK model offers a more mechanistic method that requires a great amount of nonclinical data and, for that reason, is not regularly used. [56][57]

4. Combination of PK and PD data

During this step, one of two models can be used: the threshold model or the PKPD model. The threshold model uses the human PK parameters to create PK profiles and a dosing regime in order to establish a dose concentration above the limit of efficacy (e.g. EC_{50}) and below the limit of an adverse event. On the other hand, PKPD models combine PK and PD models to create effect-time relationship, that provide the time of a given response for a specific dose regimen. This model enables the extrapolation from animals to humans based on concentration, rather than dose, which is preferable since by using concentration it accounts for PK differences. [56][57]

3.2.3 MABEL Approach

High-risk medicinal products express some concerns regarding the occurrence of serious adverse effects during FIH studies. CHMP recommends a different method to identify the safe starting dose, the Minimum Anticipated Biological Effect Level (MABEL) approach, for high-risk medicinal products that are associated with increased risk in the following factors: [58]

- Mode of Action

Several features related to the MOA can be considered in order to characterize a medicinal product as high-risk. The level of knowledge about the nature and intensity of the effect, the type of concentration-response and structure of the new drug should be considered. The pleiotropic mechanism and mechanisms that demonstrate a biological cascade or cytokine release that lead to an amplification of an effect are some examples related to high-risk drugs. [58]

- Nature of the target

The nature of the target can increase the level of risk of a new drug and the evaluation of the structure, tissue distribution, cell and disease specificity can improve the understanding of the target.[58]

- Relevance of animal species and models

A comparison between animal species and humans should be made in order to assess the relevance of animal models to study the effects of a new drug. If there are no relevant animal models, the drug is considered as high-risk. [58]

MABEL is referred as the minimum dose related to either a toxic or desired effect. In order to determine the MABEL, this approach requires *in vitro* and *in vivo* data from relevant animal models, such as target binding and RO, concentration-response curves and exposures at pharmacological doses. [56][58] The RO value must be low for an agonist (binds to a receptor and activates this receptor to produce a biological response) and high for and antagonist (binds to a receptor to oppose or reduce an action). Thus, 90% RO is appropriate for an antagonist, while <10% RO is more appropriate for agonists. However > 10% can be used for an agonist with recognized pharmacology and when there is a certain level of confidence in nonclinical data.[59]

All relevant data is scaled up to obtain human PK and PD models, that are further integrated in the PKPD modeling approach to assist in the determination of the safe starting dose. Afterwards a safety factor is applied, that must account for the criteria used to establish high-risk products. Finally, the safe starting dose derived from the NOAEL approach is compared to the one resulting from the MABEL approach; in case the resulting doses are different, the lowest dose should be used in FIH studies. [58]

3.2.4 PK Modeling

The determination of the NOAEL and AUC in multiple species is the first step of the PK modeling; the species that provides the lowest NOAEL is selected for scaling. For the calculation of the starting dose, CL and F are scaled to obtain estimates in humans. Therefore, the FIH dose can be determined by:[56]

Formula 6: $\text{Dose} = (\text{CL} \times \text{AUC}) / \text{F}$

As an alternative, the AUC obtained from a species whose CL is closest to the one predicted in humans is used. After, a correction factor is applied to control the inability of the method to account for interspecies differences in drug potency: [56]

Formula 7: $\text{Dose} = (\text{AUC}_{animal} \times \text{CL}_{human}) / \text{correction factor}$

3.2.5 Similar Dose Approach

The similar dose approach is used when the drug that is currently being tested (i) is in the same class as another approved drug (r) and when PK and PD data are very similar between those two drugs. [60]

The following equation is used to determine the FIH starting dose:[56]

Formula 8: $\text{Dose}_i = \text{Dose}_r \times (\text{NOAEL}_i / \text{NOAEL}_r)$

This approach assumes that the ratio of the starting dose to the NOAEL will be the same for both compounds. The resulting safe starting dose is characterized as a dose that will not produce any PD response. After the dose is calculated, a safety factor should be applied, in order to accommodate uncertainty. [56][60]

3.2.6 STD10 Approach and HNSTD Approach

This method has been recommended in ICH S9 to be used on small molecule cytotoxic anticancer agents, with the support of toxicity studies conducted in rodent and non-rodent species. The FIH dose should be either: [47][61]

- 1/10 of the severely toxic dose in 10% of rodents (STD10) or;
- 1/6 of the highest non-severely toxic dose (HNSTD) that does not cause lethality, life threatening toxicity or irreversible findings in non-rodents.

The first step of this approach establishes the STD10, which is later converted to human dose through scaling factors. Since the safe starting dose is one tenth of the STD10, a safety factor of 10 is used. At the same time, the HNSTD is calculated, that is later transformed into a human dose with scaling factors and with a safety factor of 6. The lowest determined dose is considered as the safe starting dose in humans. However, when the 1/10 of the STD10 provides de lowest dose, but the non-rodent is the most appropriate species, the dose derived from the HNSTD approach should be used or vice versa. [61]

The resulting dose is expected to have pharmacological effect while still being safe to use. Additionally, the FIH dose should account for the disease and its severity, since in these cases it might not be suitable to start with a lower dose and ratter with the pharmacological dose. [61]

This approach may not be applicable for other types of anticancer agents. For immune modulators with agonistic properties the MABEL approach is suggested. However, for molecular targeted agents (non-cytotoxic) there is still no consensus for which selection method to use. Yet, it is important that dose selection evaluates all toxicological and pharmacological data, to safely calculate FIH dose and to reduce the number of escalations in FIH studies. [61]

3.3 Overview of Dose Selection Methods in First-In-Human Studies

The different properties of each drug accompanied with all relevant data must be evaluated and, consequently, integrated to determine the safe starting dose. It is clear that by using several sources of data results in a higher level of confidence in the determination of the FIH dose. Estimation of the safest dose is accompanied by several challenges, that include the

extrapolation of doses from animals to humans, in addition to the fact that a particular method may be useful in one drug but inappropriate for others. Safety factors must be implemented to further ensure safety; however, these factors are generally arbitrary or empirically chosen. In fact, the lack of validation of safety factors further implies that that they are selected with no clear rationale. [52]

There is no simple method to assess the safe starting dose, which is further hampered by insufficient validation methods for the determination of the staring dose. The absence of disclosure of which method was used in previous studies is also preventing scientific evaluation. Even though the NOAEL is the most widely used method, PK and PKPD models are increasingly being used in the industry. Regardless, the method must be used on a case-by-case basis considering all the different assumptions, applicability, advantages and disadvantages:[52][56]

Table 4 Summary of Dose Selection Approaches [10][56]

FIH Dose Approach	Assumptions, Applicability, Advantages and Disadvantages
NOAEL Approach	Uses an empirical approach based on dose and is used for systemically administered drugs. Scaling can be based on body surface or on body weight. This approach is safe and easy to use; however it uses an arbitrary safety factor and does not account for interspecies differences in pharmacology activity.
PKPD Modeling	PKPD models provide a simulation of the human PK/PD-time profile, by a mathematic model. This approach accounts for interspecies differences in PK and PD and reduce the use of empirical safety factors. However, they require extensive nonclinical data and several measures to establish and validate PKPD models.
MABEL Approach	This approach assumes that starting with lowest active dose is safer that the starting with the NOAEL. Is applies to biologics and small molecules and is referred as the best option for high-risk medicinal products, such as monoclonal antibodies. Based on pharmacology rather than an empirical scaling factor. Although it requires extensive mechanistic and nonclinical data.

FIH Dose Approach	Assumptions, Applicability, Advantages and Disadvantages
PK Modeling	Accounts for PK differences between species instead of empirical scaling of dose. Uses human PK parameters, such as CL and F predicted from nonclinical data and relies on the accuracy of its prediction. It has worked well for drugs that are renally eliminated. Neglects interspecies differences in PD by assuming that concentration-effect relationship is the same in animals and in humans.
Similar Drug Approach	This approach assumes that drug candidates with similar chemical structures have similar PK and PD properties and that the ratio of the starting dose to the NOAEL will be the same for both compounds. It is simple to use and requires minimal data, however, is only applicable to very limited drugs.
STD10 Approach and HNSTD Approach	This method applies to cytotoxic anticancer agents and is easy to use. Considers toxicological data from two different species (rodent and non-rodent), however excludes interspecies differences in PK and PD.

IV – DOSE-ESCALATION SCHEMAS

FIH studies are dose-escalation studies, initiated with the safe starting dose, that are intended to determine an adequate dose for further studies. For patients, the dose is escalated until the MTD is reached. Since the MTD approach is not considered appropriate for healthy subjects, the dose-escalation must stop when a maximum exposure is reached, that must be clearly defined and justified based on available data. Dose stopping criteria are also recommended to terminate dosing to ensure safety at any part of the study (e.g. serious adverse reactions in one subject). [3]

FIH studies are, usually, single ascending dose (SAD) studies followed by multiple ascending dose (MAD) studies based on results from SAD studies. Both SAD and MAD use the practice of sentinel dosing in order to reduce the risk of a new drug. For cancer clinical studies, dose-escalation methods are divided in two different categories: Rule-Based and Model-Based Escalation Methods. The use of these methods prevents the exposure of too many subjects to subtherapeutic doses while preserving safety.

4.1 Sentinel Dosing

To ensure an appropriate conduction of the FIH study and, consequently, to ensure the wellbeing and safety of all subjects, sentinel dosing is applied within a cohort in both SAD and MAD studies. In case this method is not used, scientific justification is required.[3]

Sentinel dosing refers to the exposure of one subject to the new drug prior all subjects on the same cohort. This way only one person is administered with the drug which reduces the risks of exposing all subjects at the same time. Additionally, if the study design includes the use of placebo, another subject from the same cohort must be administered with placebo. Dosing of the rest of the subjects will only occur after a favorable safety and tolerability evaluation and review of the sentinel group. Having an appropriate time to detect any adverse events is essential and requires at least 48h. This period of time will lean on PK, PD and also if there is any level of suspicion about the product. [3][4][10]

Administration of subsequent cohorts cannot occur until all subjects of previous cohorts have been administered with the new drug or placebo and all appropriate data, such as, PK, PD and safety data are analyzed. Timing between cohorts should be followed according

to the protocol, however, some flexibility is accepted in case additional data requires longer review periods.[3]

FIH studies usually include multiple cohorts of 8 to 10 subjects randomized to either placebo or active therapy, in a 3:1 or 4:1 ratio, so that more subjects receive the active therapy and the rest placebo.[10]

4.2 Criteria for Dose-Escalation

Protocols should include how dose-escalation decisions are made and which data is used to make those decisions. The size of dose increase should consider the steepness of dose/exposure-effect or dose/exposure-toxicity curves; the steeper the curve the lower the dose increments and vice-versa. [4]

PK measurements from blood samples during the conduction of the study allow dose increase to be guided by exposure. After two dose levels it is possible to determine if exposure is proportional to dose, referred as dose proportionality, which allows the prediction of exposure at subsequent dose levels. When dose proportionality is not observed (e.g. increase in exposure higher or lower than the increase in dose) the PK is nonlinear and dose-escalation must be conducted carefully.[62]

Target saturation must also be considered, since complete inhibition or activation of targets directly affects maximum exposure and additional effects are not expected by increasing the dose. In addition, smaller dose increments are recommended for higher risk drugs. Some examples of dose-escalation are: same amount of drug per dose-escalation (x, 2x, 3x, 4x, 5x), each dose is multiplied by the same factor (x, 2x, 4x, 8x, 16x) or by the Fibonacci dose-escalation (x, 2x, 3.3x 5x, 7x, 9x, 12x).[4][52][62]

4.3 Single Ascending Dose Studies

During the conduction of SAD studies each cohort is given only one dose of the new drug and subjects within that cohort are randomly assigned to receive either the new drug or placebo. Sentinel subjects are used in SAD studies and are monitored for adverse events by tests required to assess safety (e.g. vital signs, physical examination, ECG). After the

observation period, all data is evaluated before the new drug is administered to the rest of subjects in the same cohort. Escalation of the new drug in the next cohort is initiated after all relevant information from the previous cohort is reviewed. This process will continue until pre-established safety criteria, MTD (for patients) or maximum exposure (for healthy subjects) are met. [62][63]

4.4 Multiple Ascending Dose Studies

MAD studies are conducted based on data retrieved from SAD and nonclinical studies. Since most drugs are administered repeatedly over time, there is a need to investigate safety, PK and PD associated with multiple doses of the new drug. Dosing frequency and duration are selected in order to achieve steady state of the new drug in systemic circulation for a period of time. At steady state, the concentration of the drug is in equilibrium, since drug absorption is equal to drug elimination, and, therefore, safety parameters will be efficiently monitored. Several dose levels will be studied, either at or above the expected therapeutic dose level so a safety margin for repeated dose administration can be determined. [64]

Sentinel dosing is also applied in MAD studies; for each cohort, one subject receives placebo and other receives the new drug. After a safety assessment, the rest of the cohort will receive either the drug or the placebo, according to randomization. During the course of these studies, subjects will receive a dose for several days, representing the main difference between SAD and MAD studies. Blood samples and other fluids are collected throughout the study, to understand how the drug is processed in the body, for instance to identify if accumulation occurs. The dose is escalated for further groups after an evaluation of the previous cohort. [64]

SAD and MAD studies can be performed as a single study. In SAD/MAD studies, the first cohort receives a single dose and all subjects are monitored for adverse events during a washout period. After this, the same cohort will receive multiple doses of the same dose to reach steady state. If data collected during this time is considered to be safe, the dose is escalated in the next cohort. The combination of both designs allows the reduction of the study timelines. [10][62]

4.5 Dose-Escalation Methods in Cancer Clinical Studies

FIH studies when conducted in patients aim to prevent unnecessary exposure to subtherapeutic doses, while maintaining safety and rapid escalation to a therapeutic dose. Furthermore, these studies intend to establish the MTD or the recommended phase II dose (RP2D) and schedule for efficacy testing in phase II clinical studies. Consideration should be made regarding the location of the conduction of FIH studies, since the definition of the MTD and the RP2D can be different. MTD, in the U.S, is considered as the highest dose at which ≤ 33% of patients suffer a dose-limiting toxicity (DLT), while in Europe is considered the lowest dose at which ≤ 33% of patients experience DLT. The RP2D, in the U.S. is the MTD, however, in Europe, is considered one level below the MTD. [65]

Dose-escalation methods in cancer clinical studies are divided in two types of design: [10][65]

- **Rule-Based Designs**: Patients are assigned to dose levels based on target events, such as DLT, defined as an occurrence of severe toxicities during the first cycle of cancer therapy.
- **Model-Based Designs**: Statistical models assign patients to dose levels based on an estimation of dose-toxicity curves.

4.5.1 Rule-Based Designs

The up-and-down design enables dose-escalation and de-escalation according to toxicity data in the preceding cohort, however, is not widely applied in FIH studies. Other three different designs were developed: the traditional 3+3 design, accelerated titration designs and the pharmacologically guided dose-escalation. These methods are recognized as easy to use and do not involve the use of specific software. Despite that, dose-escalation for following cohorts and the definition of the RP2D are only based on data from ongoing dose levels instead of all available data. [65]

a) Traditional 3+3 Design

This design involves cohorts of three patients and the first cohort is treated with the safe starting dose determined from nonclinical toxicity studies. After a safety evaluation, if none of the patients experiences a DLT other three patients will move on to the next dose

level. In case one of three patients experiences a DLT another three patients are added to the same dose level. The study will continue until at least two patients experience a DLT (Figure 8). [65]

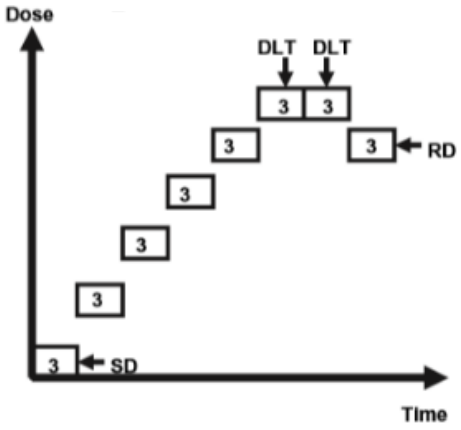

Figure 8 Traditional 3+3 Design [65]

This design is simple and safe and by adding three patients per dose level allows the assessment of PK interpatient variability. Since this method requires several escalation steps it can lead to patients being treated at subtherapeutic doses while only few are dosed with the RP2D. [65]

Several variations of the traditional 3+3 design have been proposed: [65]

- **2+4**: If a DLT is detected in one out of two patients of a cohort, extra four patients are added. The study will stop after two DLTs.
- **3+3+3**: If a DLT is observed in two out of the six patients, three more are added. When three out of nine subjects experience a DLT the study stops.
- **3+1+1**: One patient is added if one or two DLT are detected in the first three patients. One more subject is added if two DLT are experienced between the four patients. The study will end after three or more DLT.

b) Accelerated Titration Designs

Accelerated titration designs are based on one control design, design 1, and three accelerated titration designs, designs 2, 3 and 4, where only one patient is added per dose level (Figure 9). These designs are based on the National Cancer Institute Common Toxicity Criteria that define toxicity during each course of therapy as: mild (grade 1), moderate (grade

2), dose-limiting or severe (grade 3) and unacceptable, such as life-threatening (grade 4) and death (grade5). [66][67]

- Design 1 is a traditional 3+3 method with dose increments of 40% between cohorts with three new patients per dose level. Intrapatient escalation is not allowed.
- Cohorts in design 2 are comprised of one patient per dose level with a 40% dose increase between cohorts. Treatment continues until one DLT or two grade 2 toxicity are observed in the first cycle of treatment. At this stage, dose-escalation reverts to design 1 and two additional patients are added. The use of DLT elicits the switch for design 1 while grade 2 toxicity provides a supplemental safety protection element.
- Design 3 is identical to design 2, however design 3 enables the use of double-dose steps (100%) between cohorts.
- Design 4 also uses double-dose steps to allow a more rapid dose-escalation. The accelerated stage ends after one DLT or after two grade 2 toxicity during any cycle of treatment. Design 1 is implemented for further cohorts. [66]

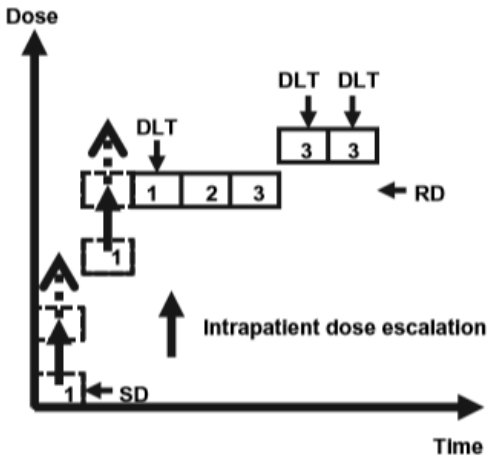

Figure 9 Accelerated Titration Design [65]

Intrapatient dose-escalation is allowed during designs 2, 3 and 4, that with the combination of the accelerated phase, reduces the number of patients treated at subtherapeutic doses and allows them to be treated at higher/more effective doses. Although evaluation of results may be difficult, since data from only one patient contributes to escalation decisions. Additional disadvantages include the fact that cumulative effects can be disguised or even difficult to distinguish from chronic/delayed toxic effects. [65][66]

c) **Pharmacologically Guided Dose-Escalation**

The first step of this method consists of the determination of a prespecified exposure from animal data. Afterwards, PK data is collected after each patient in order to determine the following dose level. Dose-escalation is based on 100% dose increments with one patient per cohort, if the prespecified exposure is not met. When plasma exposure is reached or when DLT is observed, the method changes to the traditional 3+3 design with 40% dose increments (Figure 10).[65]

This design allows more rapid dose-escalation, however, this method is not commonly used due to:[65]

- Challenges in acquiring real time PK results;
- Difficult extrapolation when dosing schedules are different;
- Increased risk of exposing patients to harmful doses if exposure obtained from the last patient was low as a result of interpatient variability.

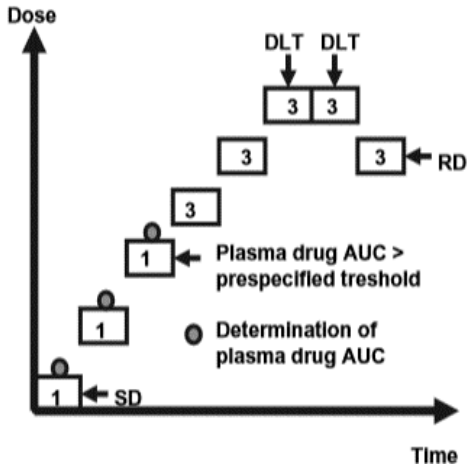

Figure 10 Pharmacologically Guided Dose Escalation [65]

4.5.2 Model-Based Designs

Model-based designs are alternatives methods for dose-escalation in FIH cancer clinical studies that differ from rule-based designs because they assume a *priori* dose-toxicity curve. Statistical models, such as Bayesian models, use toxicity data from all patients to create a dose-toxicity curve to find a dose level that generates a specific DLT. Dose-toxicity curves are described by a logistic function characterized by a parameter, θ, that represents the inclination of the curve and is adjusted based on data from all dose levels. A low θ suggests a slow increase

in toxicity and a high θ implies a rapidly increase. The dose nearest to DLT is identified and is used to treat future patients. [65]

Model-based designs unlike rule-based designs use all data from the study. However, they present some challenges, such as, access to biostatistical support and software at study site in order to adjust θ in real time. Furthermore, models may fail due to prior inadequate parameter distributions of the dose-toxicity curve. Even though the use of one patient per dose level enables the acceleration of dose-escalations, it leads to lack of data on interpatient PK variability.[65]

a) Continual Reassessment Method and Modifications

The continual reassessment method was the first proposed Bayesian method for phase I cancer clinical studies. This method assumes a *priori* dose-toxicity curve, that will be created with an initial estimate of θ, based on data from animals or similar drugs. The curve delineates the probability of the occurrence of a DLT at a specific dose, that, usually, increases with increasing doses. Investigators should prespecify an acceptable target toxicity level that will guide the selection of the dose to be administered.[65]

Once the first patient has been treated with the dose determined by the dose-toxicity curve, the toxicity outcome is observed and recorded. Such data will be statistically combined with the a *priori* dose-toxicity curve, that will change depending if the patient experienced a DLT or not. In case a DLT is observed, the curve will move to the left which indicates that the corresponding dose has higher toxicity risk; if no DLT is reached, the curve will move to the right and the dose is considered as low risk (Figure 11). This curve, as the number of patients start to increase, will become almost exclusively from clinical data. After each treatment, the dose-toxicity curve is re-estimated, and the next patient will be treated at the new best estimate of the MTD. Treatment will continue until some pre-defined condition is reached or stopping criteria is met. [68][69][70]

Some modifications to this method were developed, since if the a *priori* dose-toxicity curve and the prespecified acceptable target toxicity level is incorrect patients can be exposed to highly toxic doses: [65]

- The first patient must be treated with the safe starting dose calculated from animal data;
- The dose must be increased by one level at a time;
- No dose-escalation for the next patient if the previous one experienced DLT;

- Several patients must be treated at the same dose level, especially for higher doses and at the RP2D;
- A maximum dose increase should be prespecified to prevent overdosing, regardless the dose that is recommended by the model.

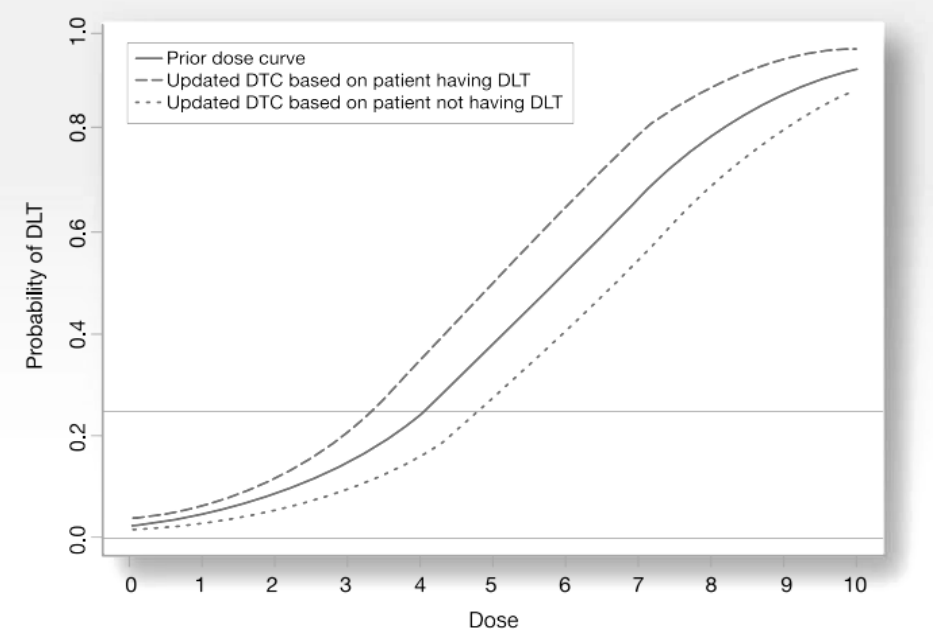

Figure 11 Theoretical Dose-Toxicity Curves for Continuous Reassessment Method [70]

b) Escalation with Overdose Control

The escalation with overdose control is a modification of the previous method that provides further safety procedures to prevent exposing patients to highly toxic doses. For every dose level, the probability of giving a dose that surpasses the MTD is assessed. If this probability exceeds a prespecified value (e.g., 25% for overdosing or 5% for excessive overdosing) the dose-escalation is stopped. [65]

4.6 Challenges in Cancer Clinical Studies

a) Delayed or Chronic Toxicities

Drugs that cause delayed or chronic toxicities can lead subjects to be treated at toxic doses before its detection. Radiotherapy is an example of anticancer treatment that causes adverse effects that only manifest after several months. Extending the monitoring period to detect these toxicities is not recommended since it would result in longer clinical studies. To overcome this problem, a modification to the continual reassessment method was created,

the time-to-event continual reassessment method (TITE-CRM). TITE-CRM estimates the dose-toxicity curve based on toxicity data from all patients. It accounts partial information and toxicity window observed so far and each patient is weighted according to how much data they provide. If subjects experience DLT, they are fully weighted, and patients that do not are weighted according to the time observed in the study. Therefore, this method allows the contribution of toxicity data for dose recommendation before the end of a follow up. This method enables shorter study durations and has been proven to be efficient in the determination of the MTD. However, the TITE-CRM assumes that toxicity remains the same through time, which may not be true. [65][71]

b) Combination Therapy

Combination therapy is widely used in cancer treatment, but it presents a challenge in FIH studies, since animal models are mainly focused on the determination of efficacy of the combination rather than toxicity. Since toxicity for each drug in a combination are already known, it is unlikely that patients will be exposed to highly toxic doses. For drugs with different MOA and no overlapping toxicities, the RP2D should be close to the RP2D of each single drug. Although, dose-toxicity curves are highly dependent on unknown PK and/or PD interactions between drugs, which can become an obstacle to the administration of the compounds at their RP2D as single drugs. PK analyses can be performed in order to understand drug interactions. First, one subject is administered with only one drug and then administered with all drugs simultaneously. This allows to compare PK properties from the administration of one drug with PK from when all drugs are administered at the same time. [65][71]

Dose levels should be selected with caution and should take into account nonclinical data and standard treatments for the intended tumor type. Rule-based designs can be used to escalate compounds to prespecified doses by: A) alternate escalation; B) simultaneous escalation, C) escalation of one agent while the other is maintained at a high dose and D) escalation of one agent while the other is hold at a low dose and then the first is reduced by one or two doses and the other is escalated to the RP2D (Figure 12). [65][71]

Model-based designs can also be used and do not require prior assumptions of the best dose combination and the dose-toxicity curve is updated after each cohort in order to establish the most active and safest dose combination. [65][71]

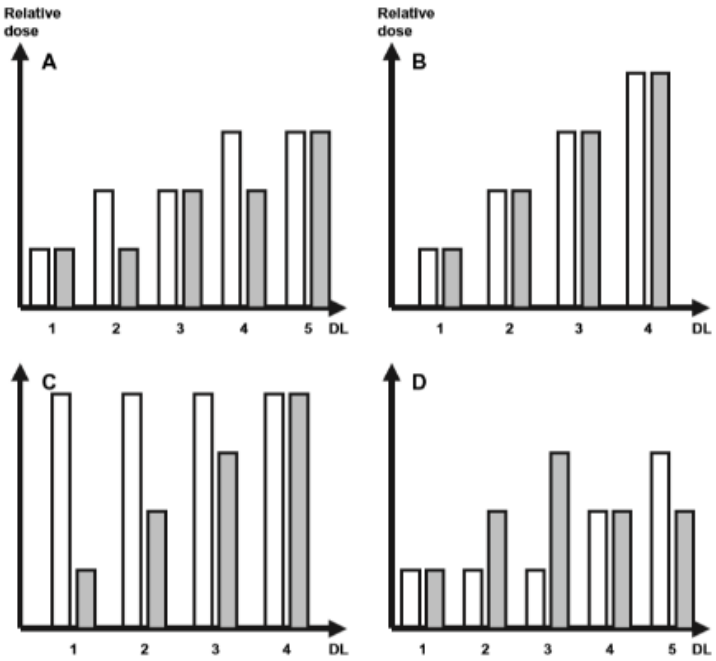

Figure 12 Dose-Escalation Strategies for Combined Therapies [65]

c) Molecularly Targeted Agents

Molecularly targeted agents block specific pathways that allow cancer cells to grow and spread, which enables less harm to normal cells. Since lower doses can be associated with higher efficacy and lower toxicity, toxicity and efficacy may not be dose dependent. Therefore, other endpoints are suggested instead of toxicity, such as:[65]

- Target inhibition in tumor/surrogate tissues: Tumor/surrogate tissues must be available and easily accessed; assays that evaluate the effect of a drug must be available and ideal target inhibition must be known. Considering that the combination of all these factors are hardly achieved, the assessment of RP2D based on this endpoint may not be recommended. The monoclonal antibody, Bevacizumab, was approved in 2004, using the traditional 3+3 design and target inhibition endpoints.
- Detection of PK levels: Plasma drug concentrations may be used, although the use of this endpoint is only appropriate if enough nonclinical data shows relevant PK-PD correlation. Transtuzumab, Imatinib, Cetuximab and Panitumumab used the traditional 3+3 design and PK endpoints.

However, most of FIH studies with molecularly targeted agents still use toxicity endpoints. No guidance has been provided on dose-escalation methods for these agents, nevertheless, efforts should be made in order to develop and explore appropriate methods for such treatment.[65]

4.7 Dose-Escalation in Recent Clinical Practice

SAD and MAD designs are systematically used in FIH studies for all types of conditions and treatments; however, specific designs were created for cancer clinical studies. Even though several model-based designs have been described, they are rarely applied in clinical practice. This comes as a surprise, since these designs treat more patients at RP2D, meaning that less subjects are treated at subtherapeutic doses. In fact, several studies in recent literature suggest that from 1991 to 2006, only 1.6% studies used model-based designs, that raised to 3.3% from 2007 to 2008, and to 6.4%, from 2012 to 2014. Despite the increase in recent years, rule-based designs are still widely used, while model-based designs are only present in a very limited number of studies due to the disregard of the advantages of model-based designs and of the drawbacks of rule-based designs.[65][72] Several barriers that prevent a greater use of these types of design were identified:[72]

- Model-based designs usually result in slower or larger studies;
- Lack of training on how to design and implement model-based designs;
- Limit resources, such as biostatical support, restrict the design and implementation of these models;
- Traditional 3+3 design is well understood and easier to implement;
- Funders influence the design of clinical studies and researchers tend to fulfill those requirements;
- Regulatory authorities are believed to be more inclined to accept studies with rule-based designs.

In order to overcome these obstacles, it is important to promote conversations between statisticians and physicians during the course of the study, so physicians can be trained and informed about study designs. Additionally, proper resources as well as practical examples to guide investigators should be available. Since funders and regulatory agencies play such a fundamental role in the selection of designs, efforts should be made to educate these entities in order to, consequently, encourage the use of better methods. [65][72]

Nevertheless, rule based-designs have proven to be beneficial and the decision of whether to use rule-based or model-based designs should be made on a case-by-case basis.

V – CASE STUDY: THE TEGENERO INCIDENT (TGN1412)

Despite strict regulatory environment and all efforts to ensure the safety and well-being of all subjects, unfortunate incidents can still happen.

TeGenero AG developed a CD28 superagonist monoclonal antibody, TGN1412 or Theralizumab, with the intention to treat inflammatory conditions. In 2006, after the conduction of nonclinical studies, that were thought to be successful, eight male healthy subjects were enrolled in the first cohort of a SAD study intended to evaluate the TGN1412. Six of these eight subjects developed severe adverse events that, ultimately, led to multi-organ failure. After this event, specialists decided to investigate the case and raised doubts about the current conduction of the drug development program. Consequently, this incident was a breaking point that led to changes in the conduction of nonclinical and clinical studies.[73][74]

5.1 Development of TGN1412

Usually, activation of regulatory T-cells (Treg cells) consists of a co-stimulatory signal from antigen presenting cells (e.g., dendritic cells or B-cells) that activate both surface T-cell receptors, by presenting the antigen through the MHC complex, and the CD28 receptors, by one of the two known natural ligands, the CD80 or CD86 (Figure 13).[75]

TeGenero created TGN1412 to modulate the activity of Treg cells. The binding of TGN1412 to CD28 receptor in T-cells leads to the stimulation of these cells without the need for T-cell receptor activation. When this happens, Treg cells release cytokines and undergo cell proliferation, increasing its number. The immune system is capable to discriminate between self and non-self, but when this process fails, cells and tissues are destroyed which leads to autoimmune diseases. For this reason, activation of Treg cells is important, since they have been shown to prevent autoimmunity.[73][76]

TGN1412 was created to treat diseases, such as, rheumatoid arthritis and B-Cell chronic lymphocytic leukemia (B-CLL), by increasing the number of T-cells, thus modulating the immune response. Regarding rheumatoid arthritis, TGN1412 activates T-cells that, consequently, reduce the levels of inflammatory mediators. In case of B-CLL, the ability of B-cells to act as an antigen presenting cells is impaired and TGN1412 can restore immune by stimulating and, therefore, increase the levels of Treg cells. [73][75]

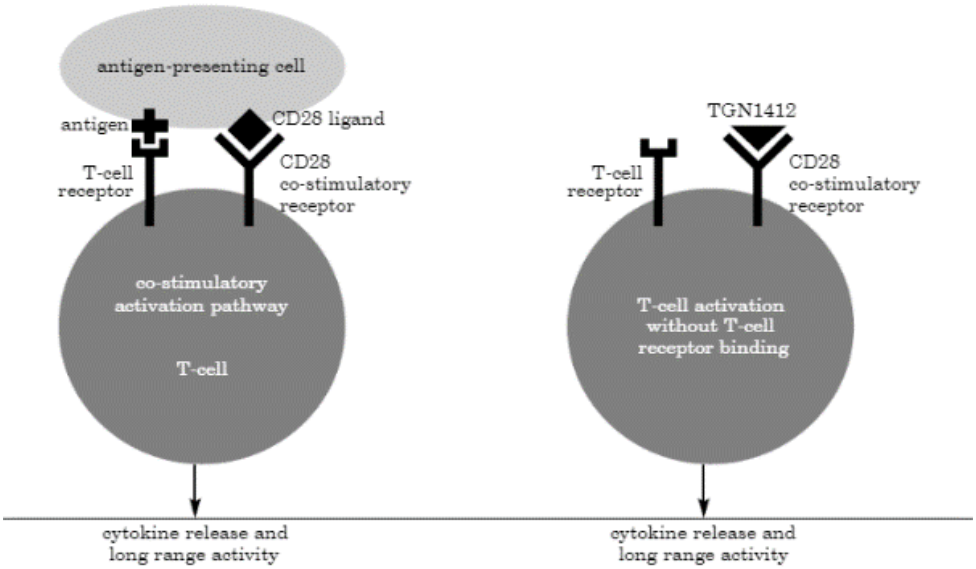

Figure 13 The Co-stimulatory Mechanism of T-cell Activation [75]

5.2 Nonclinical Studies: *In Vitro* and *In Vivo* Studies

a) *In Vitro* Studies

Several studies were performed in order to evaluate the specificity, cross reactivity and superagonistic potential of TGN1412.

Assays revealed high affinity of TGN1412 for the CD28 receptor in non-human primates (cynomolgus and rhesus monkey), although it was reported low binding affinity for CD28 receptor in rodent cells. Since CD28 proteins expressed in rhesus monkeys had the same TGN1412 binding epitope as humans, demonstrated by the difference of only one amino acid in the sequence of the TGN1412 binding site, affinity was assumed to be similar to humans. Further studies showed that TGN1412 did not cross react with other molecular targets.[75]

When conventional CD28 antibodies and TGN1412 were incubated with different T-cells from healthy donors, only TGN1412 enabled rapid proliferation of T-cells in the absence of stimulation from the T-cell receptor, proving that TGN1412 possess superagonistic activity. [73]

b) ***In Vivo* Studies**

To evaluate safety and efficacy it was essential to choose an appropriate non-human primate, such as rhesus monkeys. This selection was based on the fact that this species and humans have similar affinities for TGN1412 due to similar binding sequences. Additionally, Fc receptors and their motifs are well conserved in humans which leads to similar antibody affinities and responses. Therefore, data resulting from PK and PD studies in non-human primates was expected to anticipate drug response in humans. [73]

In a repeated dose study, doses from 5 to 50mg/mL were administered in non-human primates. It was shown that even the highest dose was well tolerated with no detection of systemic immune system dysregulation or hypersensitive reactions. Furthermore, toxicity in the CNS, CV and respiratory system was not found. In addition, animals did not present any signs of anaphylactic shock, autoimmune disease and immune suppression when treated with any dose of TGN1412, as well as, there was no indication of first dose cytokine release syndrome. Further tests regarding tissue cross-reactivity revealed consistent tissue staining in lymphoid tissue, demonstrating target-tissue specificity of TGN1412. After nonclinical studies that proved TGN1412 safety, United Kingdom regulatory authorities approved the conduction of clinical studies. [73]

5.3 Safe Starting Dose Calculation

Non-human primates that were used during nonclinical studies, were selected as the relevant model for safe starting dose calculation.

The NOAEL approach was chosen and, as a result, from repeated dose toxicity studies, the NOAEL was considered to be 50 mg/kg. By the FDA guideline, the next step was to calculate the human dose with interspecies scaling, that resulted in a HED of 16 mg/kg. After reviewing safety criteria and the application of a safety factor of 160, a dose of 0.1 mg/kg was selected as the safe starting dose for the FIH study. [56][73]

5.4 The Fist-In-Human Study of TGN1412 and Conclusions

The FIH study, initiated after the approval of the clinical study protocol, intended to evaluate the safety, tolerability and PK of TGN1412. Further objectives consisted of the assessment of the immunogenicity and effects of the monoclonal antibody on T-cells and on cytokine levels. [75]

Healthy subjects were chosen to participate since they provide comparable CD28 receptors and better immunological safety than patients. Additionally, they eliminate the effects of concomitant medication and, also, activation or dysfunctionalization of T cells due to certain diseases.[73]

Four increasing doses levels were predicted in the protocol: 0.1 mg/kg, 0.5 mg/kg, 2mg/kg and 5mg/kg, as single doses of TGN1412 administered by intravenous infusion at a predefined infusion rate. However, the study was cancelled at the first cohort due to serious adverse events in six out of eight subjects caused by cytokine release syndrome, which consists of a large and uncontrollable increase in cytokine levels. These subjects initially developed headaches, muscle pain, nausea, vomiting, diarrhea and severe fever; after several hours, hypotension, tachycardia, respiratory failure and ultimately multi-organ failure. Even though they were admitted to intensive care unit, all six subjects survived with unknown long-term prognosis. [74][75]

An investigation concluded that the TGN1412 was manufactured with appropriate excipients and with no contamination of the batch. It was also stated that the dose calculated based on the NOAEL approach was correctly administered and further procedures were in compliance with the protocol. Even though nonclinical studies did not fully assess, or the animal models did not show severe adverse events, such as immunosuppression, autoimmunity, cytokine release syndrome and implications on the CNS, the IB still registered them as possible adverse events. Therefore, TGN1412 had a severe effect in humans only detected during the FIH study, that led investigators questioning the reason to why they were absent from nonclinical studies.[75]

The IB indicated that TGN1412 had a high binding affinity for human CD28 receptor due to similar binding sequences to rhesus monkeys. However, CD28 binding sequence in humans contains a glycine in the last amino acid, but a glutamate in rhesus monkeys. This apparently small difference led to different binding affinities between TGN1412 and the CD28 receptor, caused by differences in molecular size, charge and 3D protein structure of the two

amino acids. Therefore, it was suggested that TGN1412 had lower binding affinity for the CD28 receptor in rhesus monkeys than humans, which can explain the different results between nonclinical and clinical studies.[75]

It was also proposed that even if TGN1412 had the same affinity to both CD28 receptors, the disparity of results was probably due to the interference of different proteins on the TGN1412-CD28 receptor interaction or by differences in signal transduction. Different types or similar types of cytokine that bind to differently distributed receptors or with different affinities for similar distributed receptors can produce distinct results in primates and humans. [75]

Additional justification was made about the severe consequences in clinical studies. CD28 proteins, in humans, are largely expressed on the surface of Treg cells, helper T-cells and granulocytes as opposed to monkeys. As a result, when TGN1412 bound to human CD28 receptors, the effects were amplified leading to cytokine release syndrome. Airway inflammatory responses experienced by the healthy subjects may be explained by the rapidly increase in IL-6 levels. [75]

5.5 Duff Report Recommendations

Gordon Duff, a professor of molecular medicine, investigated the TeGenero case and issued a report that included several recommendations for the conduction of phase I clinical studies in order to prevent further events. Both the conduction of nonclinical development programs and the selection of the safe starting dose are stated as the reason for the TGN1412 failure. As a result, Duff made the following suggestions: [74]

a) Conduction of Nonclinical Development Programs

All decisions about nonclinical studies and approaches used to gather relevant data should be science-based and justified on a case-by-case basis by experienced staff. While the role of several guidelines was acknowledged, their applicability to high-risk medicinal products was a concern and, for that reason, this report demanded a new guideline for these drugs. The CHMP, as a result, developed a guideline for such products, which came to fulfill that void. [74]

Duff also suggested the creation of platforms between international regulatory agencies to share data from unpublished nonclinical studies, with higher risk drugs relevant to human

safety, and from phase I clinical studies. This recommendation was based on the existence, in the European Union, of EudraCT and EudraVigilance databases, to register clinical studies and to report suspected unexpected serious adverse reactions, respectively. The creation of the EU Clinical Trials Register, in 2011, to share protocols and results from interventional studies has become helpful in the evaluation of information resulting from clinical studies. However, these databases are only for EU member states and if this recommendation was to be implemented it would cover all phases of clinical studies. [74]

More communication is suggested between sponsors and regulatory agencies in earlier stages before an application is made. However, direct communications between sponsors and review teams have decreased in the FDA and, in the European Union, communication is also difficult. The revision times for the FIH clinical study application for higher risk agents should be extended and additional opinion from independent experts should be consider when reviewing such applications. [74]

b) **Selection of the safe starting dose for FIH clinical studies**

Particular attention should be given for novel drugs, for which pharmacological activity cannot be shown in animal models or when nonclinical information is incapable of predicting human response. In this case, starting dose calculation and further increases should be proceeded with caution. It was even proposed that the calculation for safe starting dose should use all relevant information, including data regarding the novelty of the new drug, potency and MOA. Additionally, clinical studies should adopt a conservative approach in which the lowest dose calculated by different methods should be used and dosing rationale should be provided with clear justification. [74]

5.6 Strategies to Identify and Mitigate Risks for First-In-Human Clinical Studies

Rapidly after the TeGenero incident, EMA published, in 2007, a guideline to provide orientation to sponsors, stakeholders and regulatory authorities in the transition from nonclinical to clinical studies, where the MABEL approach was recommended for high-risk drugs. This guideline was revised after BIA 10-2474, a fatty acid amide hydrolase inhibitor, that led to the dead of one healthy subject in a FIH study in 2016. The "Guideline on strategies to identify and mitigate risks for first-in-human and early clinical studies with investigational medicinal products" applies to all new chemical and biological new investigational medicinal

products. Since the safety and well-being of subjects should always be the number one priority, it is important to characterize risk and create appropriate strategies to minimize that risk as well as defining a proper development program.[3][77]

Early clinical development has a certain level of uncertainty regarding the benefits and risks of a new drug which can be reduced by: quality assurance of the new drug; conduction of nonclinical testing; implementation of a scientific rationale on the selection of the safe starting dose, dose-escalation and maximum exposure and application of risk mitigation measures in the design of FIH studies.[3]

a) Quality Assurance

Proper formulation accompanied by good manufacturing practices can reduce uncertainty and further minimize the risk associated with new drugs. Determination of strength and potency must be performed in order to correctly determine the safe starting dose. Since the characteristics of the new drug can make an impact on stability, solubility and absorption to packages, it can lead to differences in exposure and bioavailability. Consequently, an evaluation about possible adsorption losses and administration systems is required.[3]

b) Nonclinical Testing

In order to minimize risk, the most important factors to be evaluated in nonclinical studies are the relevance of animal models, nature of the target, PD, PK and toxicity of the new drug. These studies must be in compliance with appropriate guidelines, such as, the ICH M3(R2) and with GLP to ensure the accuracy of results. A team of professionals integrates all nonclinical results and makes all decisions regarding the conduction of clinical studies.[3][77]

c) Dosing selection for FIH studies

Safety relies on the correct selection of the safe starting dose, which should be carefully estimated and justified. The NOAEL approach is the most used, however, other approaches can be applied depending on the level of uncertainty and the type of drug. In fact, the MABEL approach is recommended for higher-risk drugs, such as monoclonal antibodies. If this method had been used before the initiation of the TeGenero study, where no more than 10% RO was proposed, the dose would have been 0.001 mg/kg, instead of the 0.1 mg/kg calculated by the NOAEL approach. Safety factors must be applied to further mitigate the risk and their selection must be based on scientific rationale.[3][56]

d) Planning and conduct of FIH studies

Clinical studies must be designed in order to avoid exposing too many subjects and to ensure safety. FIH studies are conducted by integrated protocols, that integrate and analyze data from previous studies before making further decisions. Protocols must describe all studies activities, define stopping rules and a monitoring plan. All aspects involved in the conduction of FIH studies must be clearly justified, such as, choice of participants, route and frequency of administration, number of subjects per cohort, sentinel dosing, dose-escalation increments, stopping rules, studies sites and use of placebo.[3]

Facilities, where FIH studies are conducted, must be appropriate and prepared for emergencies. All clinical staff must be trained and experienced in identifying and responding with treatment strategies to adverse reactions. Additionally, for multicenter studies, it is crucial to define procedures to share safety data or corrective/preventive actions between sites.[3][56]

5.7 FIH Studies With Monoclonal Antibodies

In order to assess the impact of the EMA guideline, where the MABEL approach was recommended for high-risk medicines, a systematic review analyzed the dose calculation method, safety factor and the number of escalation steps used in FIH studies with monoclonal antibodies from 1990 to 2013. After an extent literature research in PubMed database, investigators identified 79 reliable FIH studies. From these studies, only 49 papers (62%) reported the method for safe starting dose calculation. In spite the fact that not all papers stated the approach, it was concluded that in recent years more studies have become transparent, which has allowed a better understanding of a given study.[78]

Most of FIH studies used the NOAEL approach (17), followed by PAD (13) and by the MABEL approach (11). Model-based approaches, such as, PKPD modeling, were the least used (8) (Figure 14). These results were not surprising, given the fact that the NOAEL is considered as an easier approach with a proper guideline, created by the FDA, to support it. Since extensive animal data is required for model-based approaches and considering that in most cases it may not be possible to gather such information to create a model, these are expected to be less used.[78]

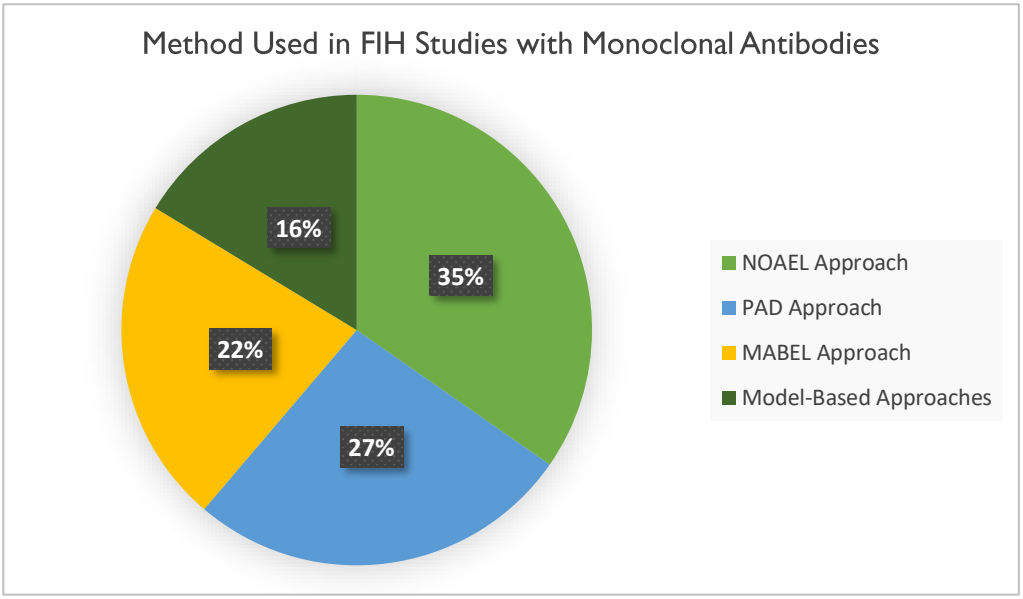

Figure 14 Method Used in FIH Studies With Monoclonal Antibodies [78]

From this research it was also possible to create an yearly trend for safe starting dose determination. The use of the NOAEL approach by FIH studies with monoclonal antibodies throughout the years has become more evident, as well as, for the MABEL approach, especially in recent years (2009-2013). However, from 1990 to 2008, studies rarely used this method (Figure 15). These results prove how the TeGenero incident and, consequently, the EMA guideline influenced the current development of FIH studies. Since EMA heavily recommends the use of the MABEL approach in high-risk medicines and also because this method uses PKPD modeling, that provides a strong estimate of the pharmacological effect of monoclonal antibodies in humans for a given dose, sponsors have been more inclined to use this method regardless of therapeutic area. In fact, the MABEL approach was used for several therapeutic areas, such as oncology, immunology and infections.[78]

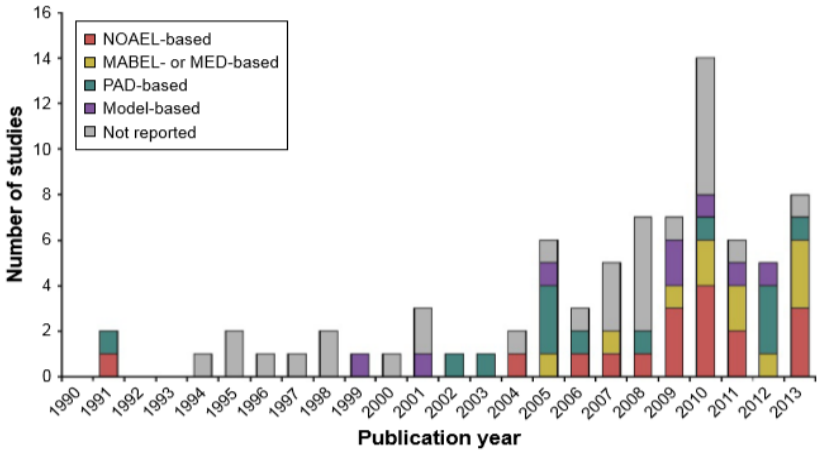

Figure 15 Yearly Trend for Safe Starting Dose Determination [78]

Regarding the use of safety factors, it was concluded that the it varied from method to method. The use of the MABEL approach resulted in lower doses than other methods which, consequently, resulted in the use of smaller safety factors. Generally, smaller safety factors correlate to a higher confidence in human safety. Since the MABEL resulted in lower safe starting doses, it was expected that more escalation steps were necessary to achieve FIH objectives. However, results show that the number of dose-escalation steps were similar across all methods, providing evidence that the MABEL approach is not inferior when compared to other approaches (Figure 16).[78]

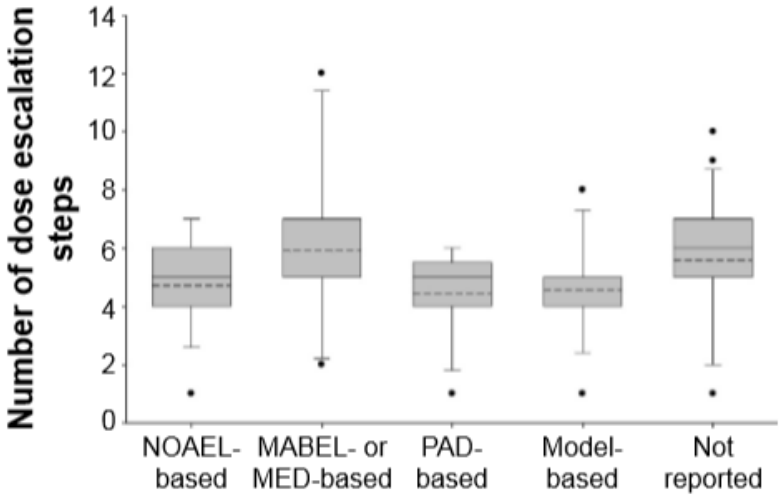

Figure 16 Number of Dose-Escalation Steps Per Method [78]

It is expected that the use of the MABEL approach will continue to grow, however, this approach is more difficult to implement when compared to the NOAEL approach. This last method is simple and easy to use that requires no modeling, which is why is constantly used. Nevertheless, it is critical to highlight the importance of the EMA guideline that is currently leading to a safer, better and more efficient conduction of FIH studies with high-risk drugs, such as monoclonal antibodies.

CONCLUSION

The transition from nonclinical to clinical studies is accompanied by major challenges. Therefore, FIH studies are extremely important, since they establish the link between animal and clinical research. However, there is a certain level of uncertainty regarding FIH studies, since the new drug is administered for the first time in humans.

Successful FIH studies require careful planning, conduction and collaboration from a variety of experienced professionals, that with the help of integrated protocols, can lead to the marketing authorization of safe and effective groundbreaking therapies.

The safety and wellbeing of subjects should always be the number one priority during clinical research. Events that have occurred with new molecules in the past, especially, the TeGenero and the BIAL incidents, that lead to the harm of several subjects, changed the conduction of FIH studies. The introduction of a set of guidelines proved to be essential to further mitigate risks associated with new drugs, in particularly with higher risk drugs and, consequently, increased the confidence about safety and the global trust in clinical research.

The selection of appropriate animal models used in these studies is extremely important, since data generated by these animals is going to be used as the starting point for clinical studies. Furthermore, toxicity data derived from animal studies plays a crucial role in the decision of dose-escalation and consequent number of dose-escalation steps used in the design of FIH studies. Therefore, the overall success of a FIH study is directly affect by how well nonclinical studies are conducted.

The correct safe starting dose calculation, that must account for the characteristics of the new drug and if it is expected to be administered in healthy subjects or patients, still requires special attention. Sponsors, usually, do not disclose which method is used, which further prevents the validation of such methods. If this information was made public, specific guidelines could be developed as well as the creation of more examples on how to apply such methods.

To further improve the conduction of clinical research, experts in this area must encourage information sharing and share their knowledge and opinions to inform those with less experience.

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