PRECLINICAL REGULATORY APPROACH BEFORE CLINICAL DEVELOPMENT AND MARKETING AUTHORIZATION OF MEDICINAL PRODUCTS IN THE EUROPEAN UNION

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ABSTRACT

The non-clinical assessment first marketing approval of a pharmaceutical in the European Union mainly includes several recommendations developed in the International Council for Harmonisation (ICH) and European Medicines Agencies (EMA) guidelines. The recommendations in ICH guidelines further harmonize the non-clinical studies among the regions of the European Union (EU), Japan and the United States. These guidelines represent the consensus reached regarding the type and duration of non-clinical studies to support human clinical trials and marketing authorization for pharmaceutical products. More specifically, in the EU, the rationale and requirements for non-clinical testing in the development of medicinal products for human use are defined in Directive 2001/83/EC as amended. UE directive and EMA guidelines should be read in conjunction with ICH guidelines before applying for a clinical trial or marketing authorization in the EU. The Directive 2010/63/EU also should be considered to perform non-clinical studies taking into account the protection and welfare of animals used for scientific purposes. The main goals of the nonclinical studies generally include a characterization of pharmacology, pharmacokinetics, toxic effects concerning target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility. These data should help to define the estimated therapeutic dose, the maximum dose, and dose steps and intervals for clinical trials in humans. The non-clinical studies recommended to support marketing authorization are conducted all along the process; hence, the requirements that must be satisfied are different for each phase. The guideline ICH M3 (R2) delivers practical recommendations for timing or when to conduct which non-clinical studies. Specific consideration should be taken into account for anticancer drugs and biotechnology-derived pharmaceuticals. We have summarized the main non-clinical studies required before clinical development and marketing authorization in the European Union.

Nonclinical studies

1. **Pharmacology studies**
   - 1.1 Primary and secondary pharmacodynamic
   - 1.3 Safety pharmacology studies.
2. **Pharmacokinetics and toxicokinetics studies**
3. **Toxicity studies**
   - 3.1 Single and repeated-dose toxicity studies
   - 3.2 Genotoxicity studies
   - 3.3 Carcinogenicity studies
   - 3.4 Reproduction toxicity studies
   - 3.5 Other toxicity studies
4. **Environmental Risk Assessment**

Timing of nonclinic al studies (ICH M3 (R2))

Laboratory phase of pharmaceutical development → Phase I → Phase II → Phase III → Marketing authorization

Before Phase I

Pharmacology:
- *-Pharmacodinamic studies*
- *-Safety pharmacology:*
  Cardiovascular , Central nervous and Respiratory system.

Pharmacokinetics:
- Systemic exposure data
- In vitro metabolic
- Plasma protein binding

Repeated dose toxicity: different duration depending clinical trial. (evaluation of reproductive organs)

Genotoxicity studies. In vitro studies

Phototoxicity Evaluation of photochemical properties *****

Before Phase II

Genotoxicity studies:
Standard test batery for genotoxicity

Before Phase III

Pharmacokinetics:
In vitro potential drug interaction
Absorption
Distribution
Metabolism
Excretion

Reproductive toxicity studies:
fertility
embryotoxicity
Pre-and postnatal development

Photosafety (if necessary) *

Before Marketing

Pharmacology:
- **Safety pharmacology**
  Follow- up and supplemental safety pharmacology studies.

Repeated dose toxicity: different duration .

Carcinogenicity

Environmental risk assessment

Other toxicity studies (if necessary)
Inmunotoxitity, local tolerance, juvenile animal studies …)

Anticancer pharmaceuticals – ICHS9

The nonclinical data to support Phase I and the clinical Phase I data would normally be sufficient for moving to Phase II.

Genotoxicity

These studies are not considered essential to support clinical trials, but genotoxicity studies should be performed to support marketing.

Carcinogenicity

Carcinogenicity studies are not warranted to support marketing for therapeutics intended to treat patients with advanced cancer.

Reprotoxicity

Pre- and postnatal toxicology studies is generally not warranted to support clinical trials or for marketing of pharmaceuticals for the treatment of patient with advance cancer.

Liposomal Products

A complete evaluation of the liposomal product is not warranted if the unencapsulated material has been well characterized. As appropriate, the safety assessment should include a toxicological evaluation of the liposomal product and a limited evaluation of the unencapsulated pharmaceutical and carrier.

Safety Pharmacology and pharmacodynamic Studies (ICH S7A)

Primary pharmacodinamic studies are intended to investigate the mode of action and/or effects of a substance in relation to its desired therapeutic target. These studies might include, among others:
- •Target interactions, receptor binding and occupancy, inhibition of enzymes, cellular response
- •Duration and (ir)reversibility of effect
- •Dose-response relationships
- •Physiological turn-over of the target

Safety Pharmacology Studies

1)To Identify *undesirable pharmacodynamic properties* of a substance
2) To Evaluate *adverse pharmacodynamic and /or pathophysiological effects* of a substance.
3) To investigate the *mechanism of the adverse pharmacodynamic effects* observed.

Safety pharmacology core battery: Cardiovascular, Central nervous, Respiratory systems.
Supplemental and follow-up safety pharmacology: When potential adverse effects raise concern for human safety then should be explored in follow-up or supplemental studies.

Pharmacokinetics (ICHS3B) and Toxicokinetics (ICH S3A) Studies

Pharmacokinetics

The study of the fate of the active subtance within the organism.

Parameters

- •In vitro metabolic / Plasma protein binding
- •Absorption (AUC, Cmax), Distribution, Metabolism and Excretion
- •Potential drug interactions

Toxicokinetics

To describe the systemic exposure (AUC / Cmax) achieved in animals, and its relationship to dose level and the time course of the toxicity studies.

Parameters

- •Plasma (or whole boold serum) concentration for the parent compound and /or metabolite.

Nanomedicines –EMA Reflection papers-

Development of block-copolymer-micelle medicinal products

Pharmacodynamic ← **Block copolimer** → Pharmakinetics

The non-clinical studies should include demonstration of pharmacodynamic response. Changes in the Pharmacodynamic and safety can occur. Moreover, it has been noted that certain **block copolymers** (*not containing an active substance*) **can display biological activity** which have an impact on clinical efficacy and / or safety.

Significant changes can occur when an active substance is administered as a block copolymer micelle product (i.e volume of distribution, clearance, half-life, etc.)

The following parameters specific to block copolymer micelle should be assessed: Cmax, half-life and AUC of the block copolymer micelle product both for the total active substance and for free active substance in blood, plasma or serum.

Genotoxicity Studies (ICH S2R1)

Genotoxicity studies are non-clinical tests designed to detect compounds that induce genetic damage.

Genotoxicity before Phase I – In vitro assays -see ICH M3(R2)-. .

Genotoxicity before Phase II - Standard test batery for genotoxicity.

a) Option 1
   i. A test for gene mutation in bacteria
   ii. A cytogenetic test for chromosomal damage
   iii. An in vivo test for genotoxicity, generally a test for chromosomal damage using rodent hematopoietic cells

b) Option 2
   i. A test for gene mutation in bacteria
   ii. An in vivo assessment for genotoxicity with two different tissues

Single dose toxicity – ICH M3

Single-dose toxicity may generate useful data to describe the relationship of dose to systemic exposure and /or local toxicity. These data can be used to select doses for repeated dose toxicity studies.

Non clinical studies shall be carried out in two species of mammals. The use of one species is acceptable if it has been unequivocally demonstrated that other available species are irrelevant as models for human safety assessment. Also, acute toxicity studies are considered to be of very limited value (if any) for predicting consequences of overdose in humans.

Repeated Dose Toxicity (EMA CPMP/SWP/1042/99 Rev. 1 Corr.)

Objectives

To characterize the toxicological profile of the test compound following repeated administration. This includes identification of potential target organs of toxicity and exposure / response relationships, and may include the potential reversibility of toxic effects.

Monitoring during the study

- •*food intake, general behaviour, body weight, haematological parameters, clinical chemistry, urinalysis and ophthalmology.*
- •*electrocardiographic recordings should be obtained in non-rodent species.*
- •*toxicokinetics parameters.*
- •*autopsy and hystopathology examination.*

Recommended Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials:

Maximum Duration of Clinical Trial	Minimum Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials	
	Rodents	Non-Rodents
Up to 2 weeks	2 weeks	2 weeks
Between 2 weeks and 6 months	Same as clinical trial	Same as clinical trial
> 6 months	6 months	9 months

Recommended Duration of Repeated-Dose Toxicity Studies to Support Marketing

Duration of Treatment	Rodent	Non-Rodent
Up to 2 weeks	1 month	1 month
>2 weeks to 1 month	3 months	3 months
>1 month to 3 months	6 months	6 months
>3 months	6 months	9 months

Carconogeninicity Studies - ICH S1A

Objectives

To identify a tumorigenic potential in animals and to assess the relevant risk in humans.

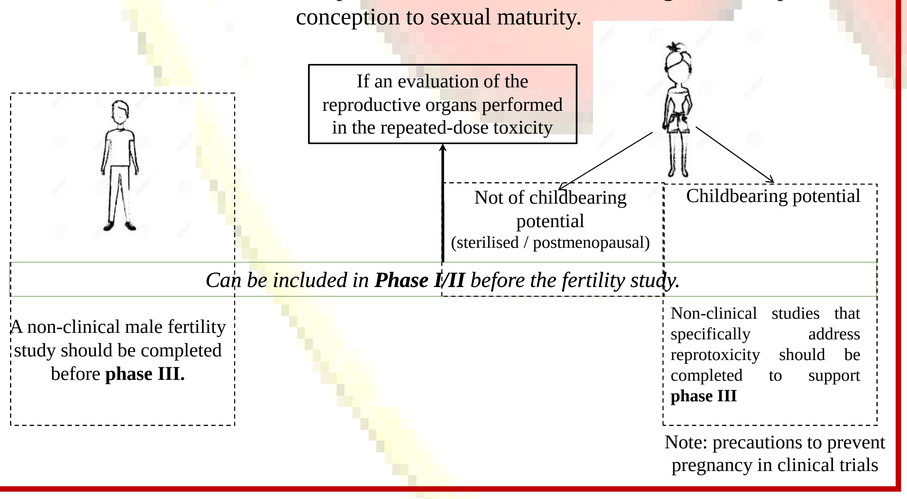
Carcinogenicity studies

- •Should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months (continuously or intermittently).
- •Compounds administered infrequently do not need carcinogenicity studies unless there is cause of concern as:
  - Previous demonstration of carcinogenic potential in the product class
  - Structure-activity relationship suggesting carcinogenic risk
  - Evidence of preneoplastic lesions in repeated dose toxicity studies
  - Long-term tissue retention of parent compound.

Reproduction Toxicity Studies -ICH S5 (R2)

Objectives

The aim of reproduction toxicity studies is to reveal any effect on mammalian reproduction. The studies selected should allow exposure of mature adults and all stages of develpment from conception to sexual maturity.



Biotechnology-derived pharmaceuticals -ICH S6 (R1)

It applies to products derived from characterized cells through the use of a variety of expression systems including bacteria, yeast, insect, plant, and mammalian cells. The active substances include proteins and peptides, their derivatives and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology.

Preclinical safety testing should consider:

- •Selection of the relevant species, age and physiological state
- •The manner of delivery-dose, route, administration, treatment regimen.
- •Stability of the test material under the conditions of use

Biological activity / Pharmacodinamics

For monoclonal antibodies, the immunological properties of the antibody should be described in detail, including its antigen specificity, complement binding, and any unintentional reactivity and/or cytotoxicity towards human tissues.

Specific approach

Repeated toxicity studies

For biopharmaceuticals intended for short-term use (e.g., < to 7 days) and for acute life-threatening diseases, repeated dose studies up to two weeks duration have been considered adequate to support clinical studies as well as marketing authorization.

Genotoxicity Standard test are not applicable. Test should be performed in those cases where there is a cause of concern.

Carcinogenicity. Standard carcinogenicity are generally inappropriate. However, product-specific assessment may still be needed depending upon duration of clinical biological activity of the product.

Requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product

Iron-based products used to treat iron deficiency consist of a polynuclear iron core, generally present in the iron (III)-oxyhydroxide form, stabilized by a carbohydrate complex which leads to nano-sized colloidal structures. These complexes will be internalized by cells via the endocytic route. Localization of iron-based products to liver macrophages or hepatocytes has been noted after intravenous administration.

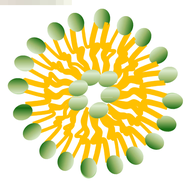
Table 1. Relevant compartments for the distribution of intravenous iron- based nanoparticles for iron deficiency

1. Plasma (or serum) and red blood cells
2. RES: macrophages
e.g. in spleen, liver (Kupffer cells)
3. Target tissues
3.1 Pharmacological target tissues
e.g. bone marrow
3.2 Toxicological target tissues
e.g. kidney, liver (hepatocytes), lungs, heart

Toxicity studies are not sensitive enough to demonstrate differences between test and reference product. Therefore, they are not useful for this purpose In case of specific safety concerns, appropriate safety endpoints included in the design of the bio-distribution study may be sufficient to address these concerns.

Requirements for intravenous liposomal products developed with reference to an innovator liposomal product

Pharmacodynamic where possible the development of in-vitro tests capable of characterizing any interaction between liposomes and target cells



Pharmakinetics The complete characterization of the stability, pharmacokinetics (including tissue distribution) of a new liposomal product is critical to establish safe and effective use.

Liposomal medicinal products have formulation and manufacturing-specific distribution characteristics after intravenous administration and similar plasma concentrations may not correlate with therapeutic performance. variation in production and product and process control technology can lead to products with different therapeutic performance.

GENERAL CONSIDERATIONS

3Rs *(Replacement, Reduction and Refinement)* needs to be considered when selecting testing approaches to be used for regulatory testing of human and veterinary medicinal products in line with Directive 2010/63/EU.

GLP In general, studies should be carried out in conformity with the provisions relating to good laboratory practice (GLP).

Relevant species. The demonstration of the relevance of the animal model(s) may include a comparison with humans of Target expression, distribution and primary structure.
- • Pharmacodynamics; Metabolism and other PK aspects;
- • On- and off-target binding affinities and receptor/ligand occupancy and kinetics.
- • In vitro metabolic profile. (exposure to main human metabolite should be ensured)

Safety Pharmacology studies can be integrated in repeat dose toxicity studies

Pharmacokinetic / Toxicokinetic. Sponsors should supply a brief summary of the analytical assays used to characterize the non-clinical PK and TK, including their accuracy, precision and limits of quantification.

Nanomedicines. Any variation in mean/median size and size distribution and /or the accuracy of methods employed for nano-sizing may result in the generic product displaying different physicochemical properties leading a different biopharmaceutical profile in respect of pharmacokinetics and biodistribution.

Coating nanomedicine products The coating is used to minimize aggregation and improve stability. A coating has the potential to impact the safety and efficacy. It should be considered the effect of the coating on the product stability, pharmacokinetics and biodistribution, etc.

Route of administration The expected clinical route of administration should be used.

References

AEMPS Spanish Agency of Medicines and Medical Devices *https://www.aemps.gob.es/home.htm* ***/ EMA European Mediciens Agency*** https://www.ema.europa.eu/en; ***ICH***International Council for Harmonisation https://www.ich.org/home.html

ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals
ICH S1A Need for Carcinogenicity Studies of Pharmaceuticals
ICH S2 (R1) Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use
ICH S3A Note for guidance on Toxicokinetics
ICHS3B Guidance for repeated dose tissue distribution
ICH S4 Duration of chronic toxicity testing in animals (rodent and non-rodent toxicity testing
ICH S5 (R2) Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility
ICH S6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals
ICH S7A Safety pharmacology studies for human pharmaceuticals
ICH 29 Nonclinical evaluation for anticancer pharmaceuticals
CPMP/EWP/560/95/Rev. 1 Corr. 2** Guideline on the investigation of drug interactions
CPMP/SWP/1042/99 Rev 1 Corr* Guideline on repeated dose toxicity
EMA/CHMP/CVMP/JEG-3Rs/450091/2012 Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches).
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EMA/325027/2013. Reflection paper on surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products.