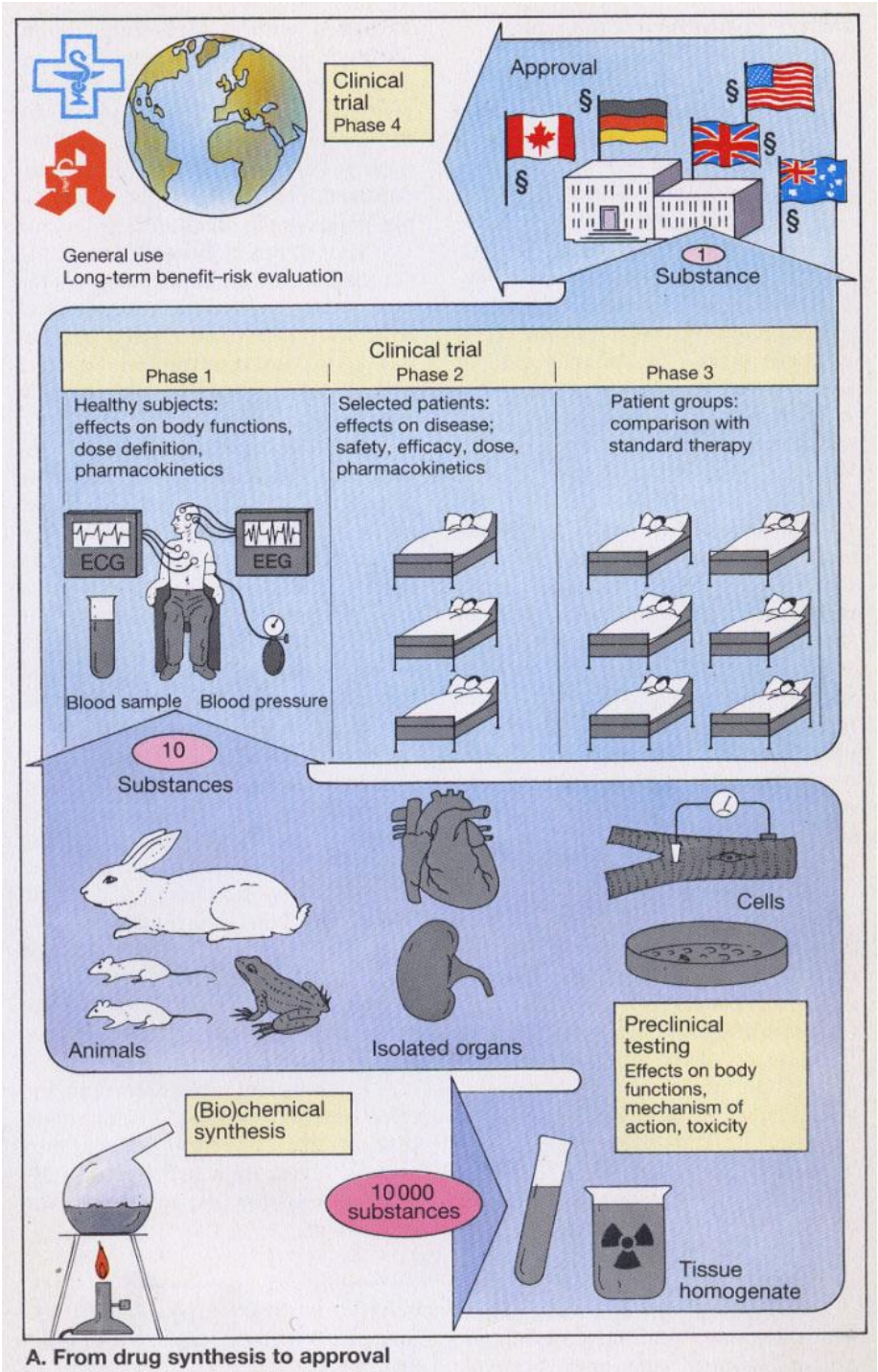
Introduction to experimental pharmacology: from in vitro to in vivo studies

Research and development in pharmacology

- Preclinical studies
- Cinical trials

Drug R&D : evolve through steps which take in consideration all the informations available at that stage of the study



How is a drug born?

Conceptual research (natural substances, traditional medicine, drug design,…)

Casuale discovery of a new substance (*serendipity*)

Talidomide (N-ftalimido-glutaride) history

Immunomodulator with antiangiogenic proprerties

- 1950 borns as sedative and hypnotic
- Prescritpion in the control of nausea in pregnancy in the 60s
- 1961 first report of focomelic newborns (10000) (Lancet)
- Drug withdrown
- 1965 use in leprosy male patients for hypnotic use, effective within 4-48 h
- 1989 starts the use as immunomodulator in transplants
- 2000 use in HIV & cancer

Other examples…

- Minoxidil (vasodilator *via* K-channel) designed as antihypertensive drug

  used ad drug against alopecia

- Sildenafil (phosphodiesterase inhibitor) designed as anti-angina drug

  used for erectile dysfunction/impotence

Conceptual research and discovery of new susbtances

- 1 – 2 years
- Approx 8000 substances potentially active

Research aim

Define and synthetize new susbtance

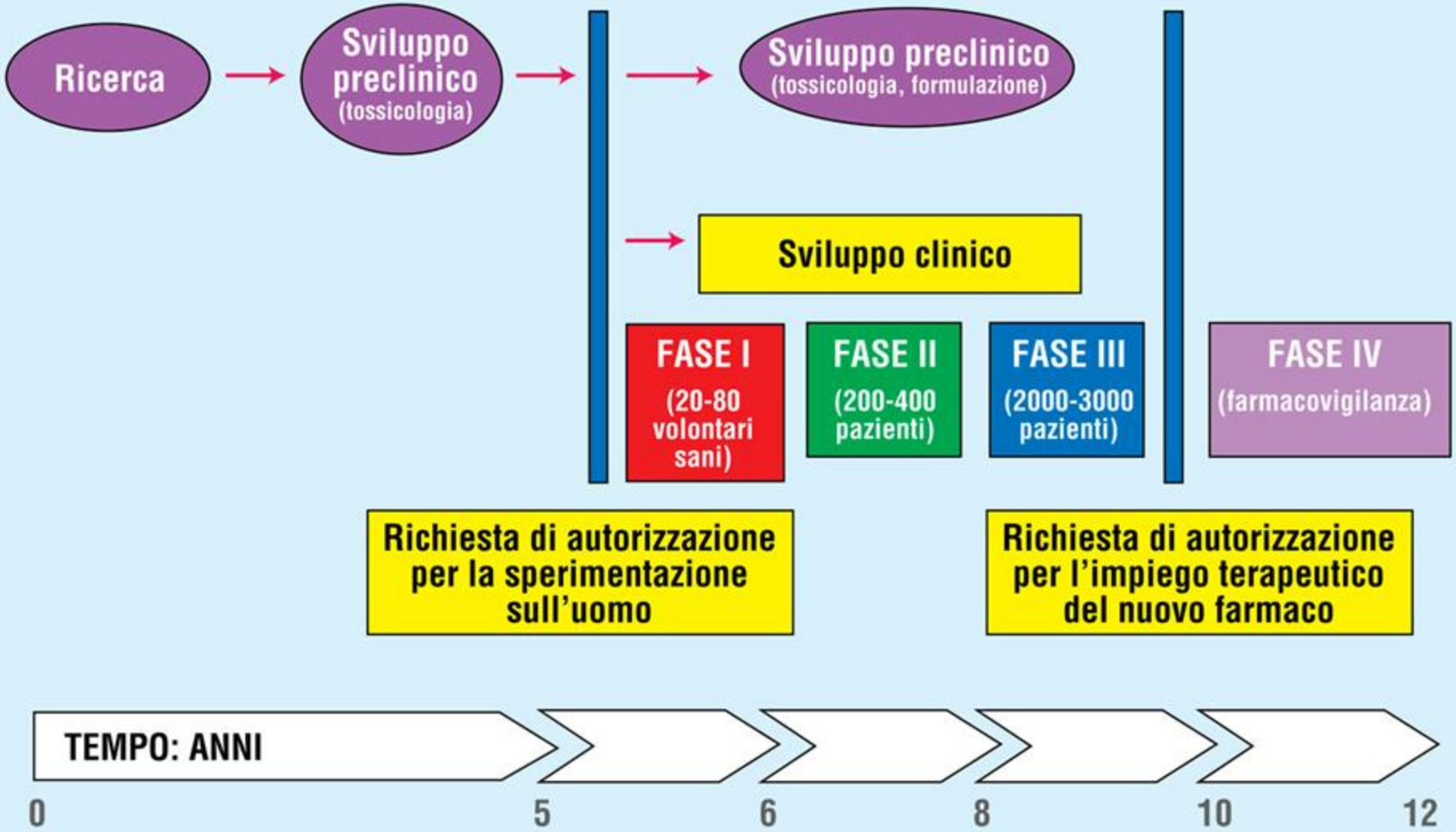
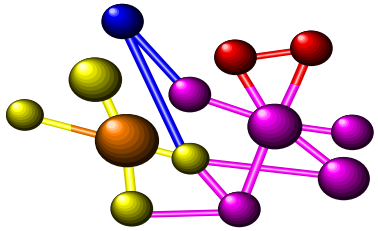


Fig. 18.1. – Sviluppo di un farmaco, dalle fasi di ricerca e scoperta a quelle di sviluppo clinico.

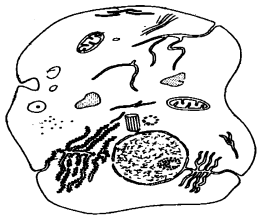
How?

- Hypotehsis and ideas
- Synthesis on lab scale of the susbtances



- Screening on cells/tissue of its future indication
- Evaluation and validation in aniaml models

PRECLINICAL STUDIES



- Durantion: 2-3 y

Select from thousands screened substances

20 – 30 molecules pharmacologically and biochemically interesting

Chemical structure correlation with specific pharmacological action (SAR studies) resulting in further reduction of the number of molecules under investigation

Preclincal pharmacological research

◎ development of potential new drugs

- Pharmacological tests to screen, understand the mechanisms of action, receptor activity and biological efficacy of the new molecules
- Toxicity and pharmacokinetic studies
- Choice of the drugs entering into the clinical trials

TIME: 5-6 y

Pharmacological studies

- Different in relation to the potential mechanism of action and the class of drugs in development
- Screening/comprehension of the mechanisms
- In vitro, ex vivo & in vivo (experimental animals)

EXPERIMENTAL MODELS to DEFINE the PHARMACOLOGICAL PROFILE of a COMPOUND

1. MOLECULAR LEVEL
    - A. Receptor binding
    - B. Enzymatic activities
2. CELLULAR LEVEL
    - A. Cell cultures
    - B. Isolated tissues *(vessels, heart, intestine etc..)*
3. ANIMAL MODELS
    - A. Normal animals
      (mouse, Rat, dog, cat, rabbit, monkey)
    - B. Animal models reproducing the disease

Cellular models and the preclincial development of drugs

1. **Primary cell cultures form human and animal source** *(neurons, hepatocytes, etc)*
2. **Cells transfected with the proteins target of the drug action** *(HEK 293, CHO transfected with receptors, ion channels, ttransportes, enzymes)*
3. **Tumor cells**

ANIMAL MODELS REPRODICING the DISEASE CHARACTERISTICS and DRUG EVALUATION

1. **Transgenic mice:** Animals in which an exogenous gene *(transgene)* normal or mutated, is added to the genome and expressed in a specific tissue
2. **"Knockout" mice:** Animals in which specific genes are mutated or made inactive *(Parkinson, Alzheimer, homozigous for ob gene)*
3. **Animal Genetically expressing a pathology** *(hypertensive rats , obese mice, ...)*
4. **Animals treated with strategy/molecule which reproduce the disease state** *(rats with flogistic sites, rats with ulcers, diabetic rats, ...*

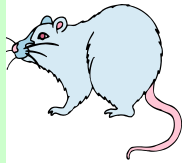
IN VITRO (examples)

- Enzymatic activities (enzymes isolated from tissues or cells) to study inhibitors/activators
- Electrophysiology techniques (blockers/activators of ionic channels)
- Receptor Binding
- Enzymes/second messengers involves in signal transduction
- Specific cellular functions on normal or transfrmed or genetically modified cells

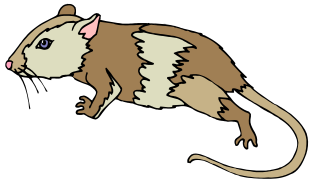
EX VIVO (examples)

- Tissues , assessement of integrated responses
- Tissue slices (es. brain)
- Isolated Organs (preparations from the heart, vessels, intestinal muscles, excretory system, reproductive organs)

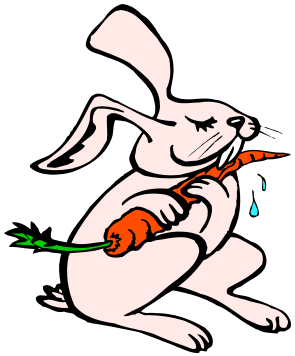
Animal models (IN VIVO)



mouse

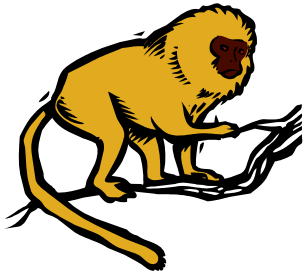


Rat



rabbit

dog



Primate

Essential documents for preclinical study

- Protocol (according GLP)
- Study results
- Ethycal clearance (local and national commetee)

PRE-CLINICAL STUDIES

PHASE I

PHASE II

PHASE I

Detailed Pharmacology studies to assess

- ✓ The main therapeutic effects
- ✓ Side effects
- ✓ Acute **toxicity**
- ✓ Solubility of susbtance

PHASE II

AIMS

1. pharmacokinetic/dynamic profile

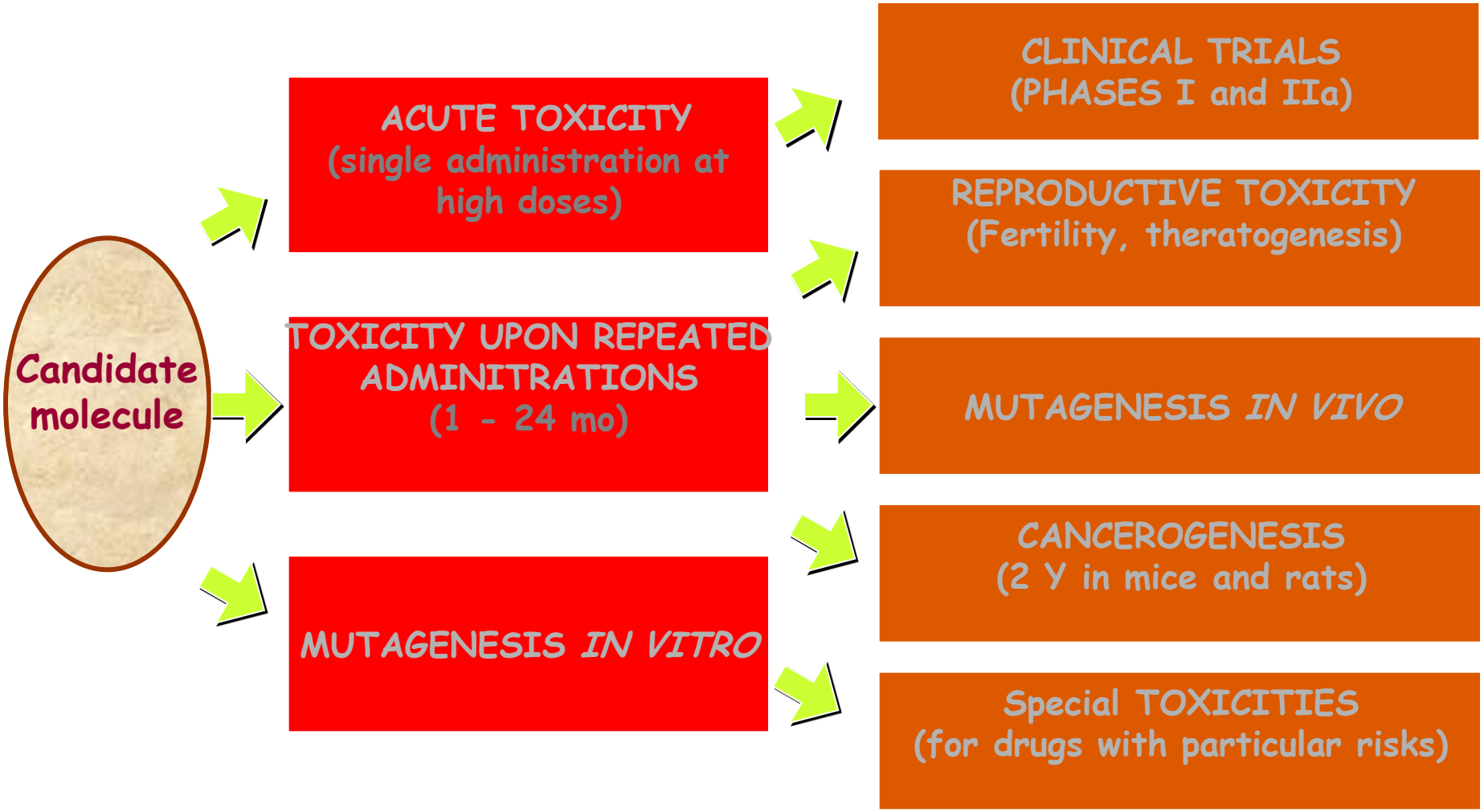
2. Subacute and chronic toxitity

3. **Toxicology of reproduction**

4. **Mutagenesis & cancerogenesis**

5. Stability

Toxicological studies to assess the safety of a new drug



Required elements for a toxicological diagnosis

- Clinical signs, body weight, food and water consumption
- Haematolgial exams, clinical analysis
- Ophtamologic and veterinary examinations
- Cardiovascolar (ECG), neurologic (spontanoeus motility, behaviour)
- Organ modification : macroscopic and microscopic inspection (autopsia)

Preclinical results are used for:

- Synthesis of active susbtances at "pilot scale"
- Possibility of scale up synthesis
- Defintion of the final galenic form
- Evaluation of stability of the final pharmaceutical formulation
- Scale-up synthesis for clinical trials

HYPOTHESIS IDEA

Chemical-pharmaceutical data

Laboratory animals

Pharmacodynamic data

Toxicology data

Pharmacokinetic data

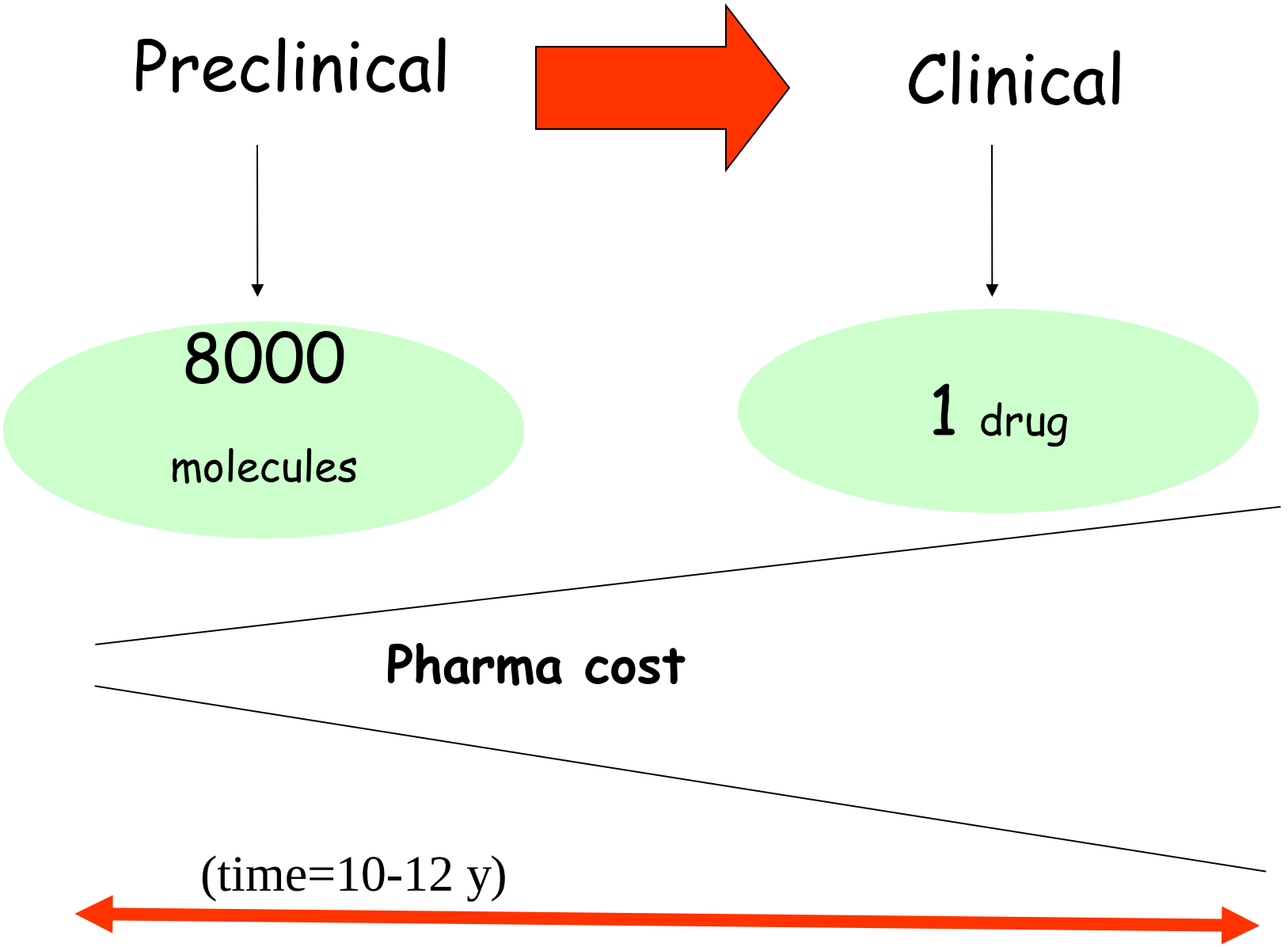
Ethycal and clinical evaluation

Ethycal and strategic criteria to start clinical trials

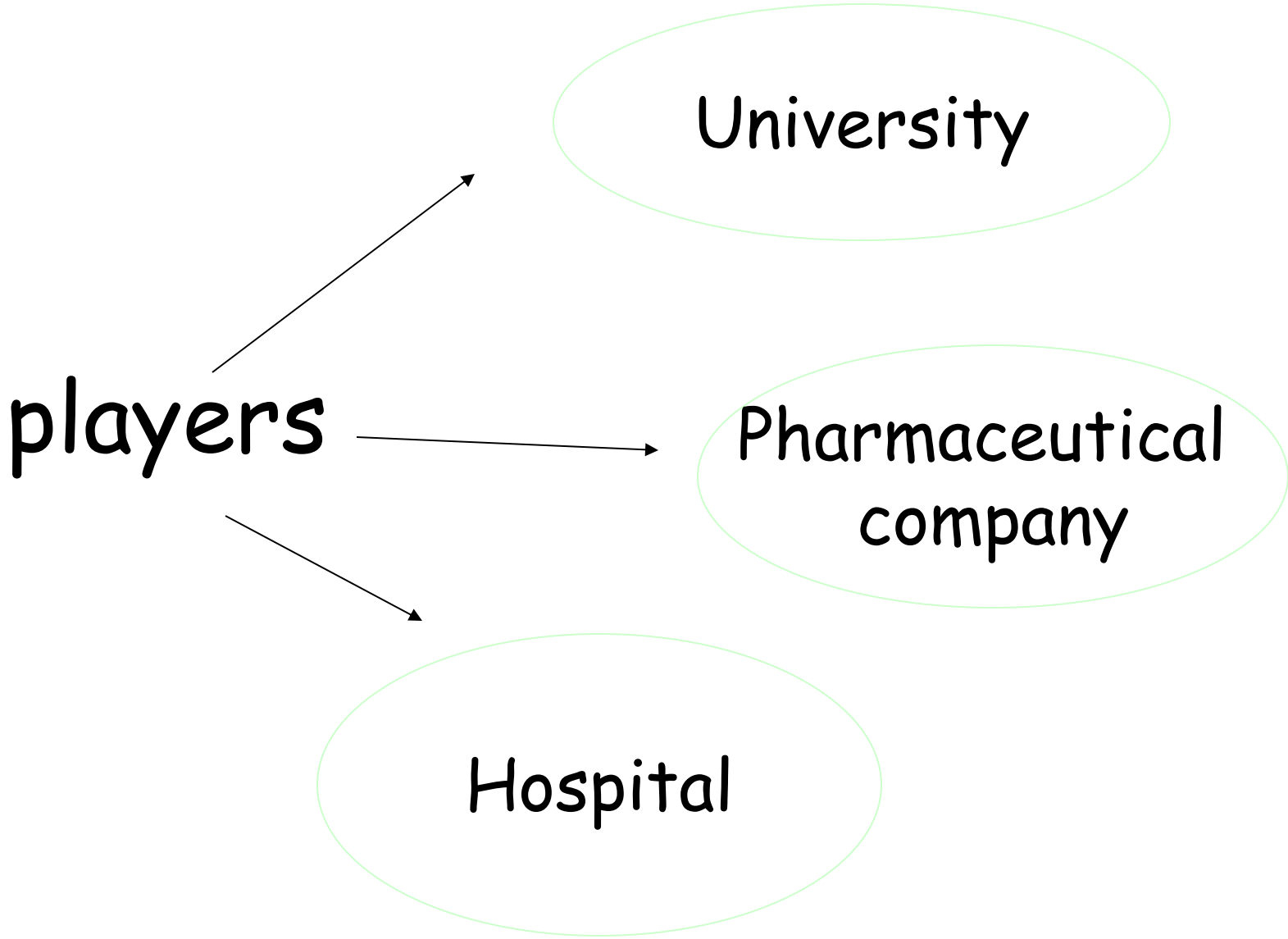
- Evaluation benefits/risks
- Possibility to transfer the pharmadynamic data obtained on the animal to humans
- Low risk for human in relation to

Potential benefit

Urgency to have the new drug


Preclinical
Clinical
8000
molecules
1 drug
Pharma cost
(time=10-12 y)

Clinical studies on humans


University
players
Pharmaceutical company
Hospital

Project manager

Clinical Research Associate

Forensic dept

Biostatistic dept

Pharmaceutical company

Finantial dept

Pharmacovigilance dept

Data Management

Production

Quality Assurance

Hospital

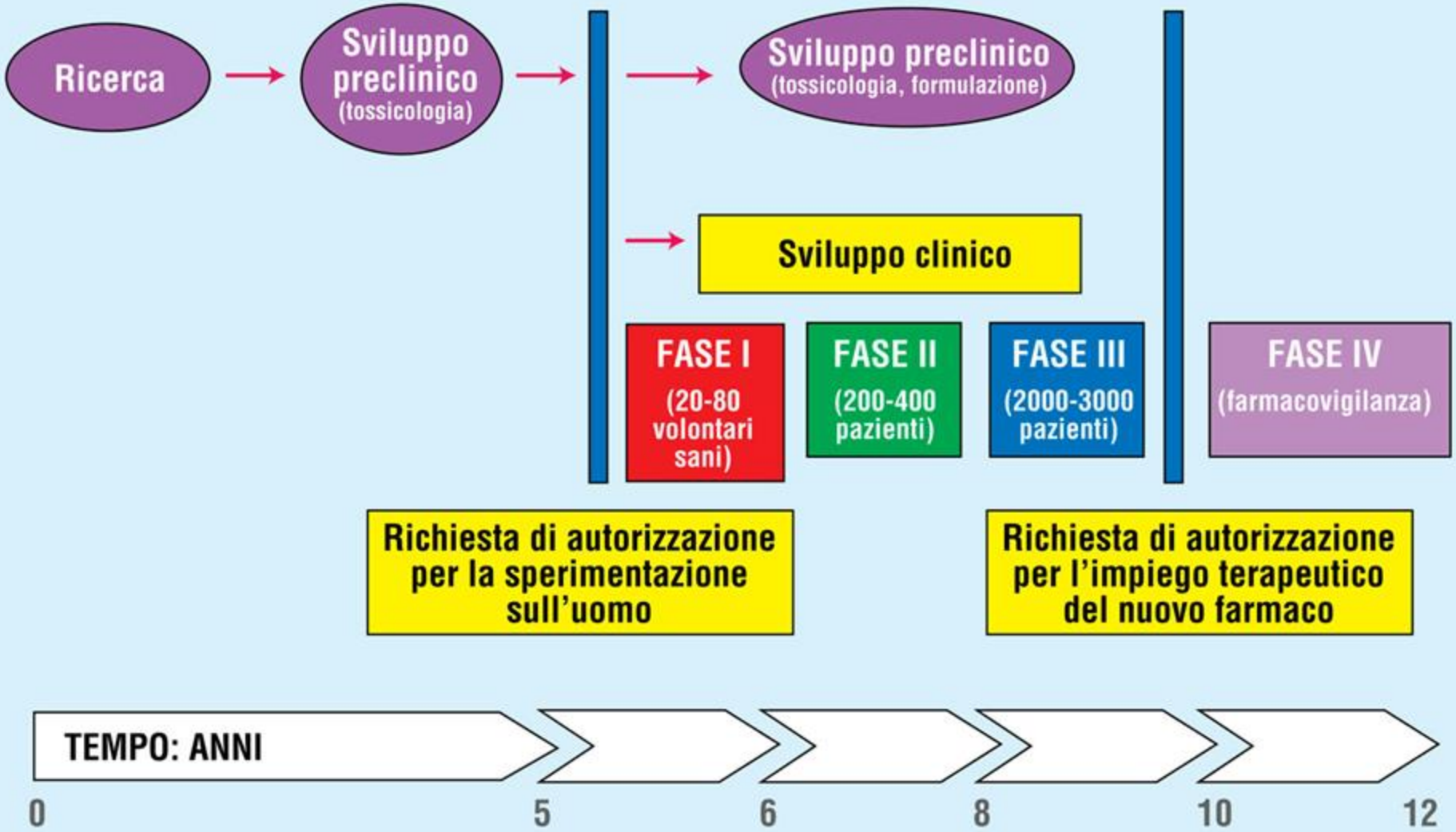
Ethyical cometee

Sanitary direction

University

Pharmacy

Medical doctors



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Clinical trials

- Phase I: healthy individuals/diseased individuals
- Phase II: drug doses and administration routes
- Phase III: drug efficacy
- Phase IV: post marketing

Document requirements to start a clinical trial

- Protocol
- Investigator brochure (IB)
- Clinical data record Form (CRF)
- Information for the subject
- Informed consent form
- Contract
- Insurance
- Autorization (CE)

Phase I

- Generally on healthy subjects
- Limited numeber of participants (20 - 50)
- Short duration
- Allows to define three maxiamally tollerated doses, time of administration in relation to the pharmacokinet data, galenic form

Phase II

- Patients affected by the pathology
- Limited number of patients for a short period
- Allows to define the best dose regimen
- The pharmacological activity can be compared to the placebo or a reference drug

Phase III

- Patients with the target pathology
- Large numbers of patients
- It allows to define the efficacy of the drug
- time……..

Phase III

- time….. depends on the pathology and the reference treatment

- Drug for Depression: 4w – 6 m
- Drug for Hypertension: >3 – 6 m
- Drug for Diabetes: 6 m – 1 y
- Drug for Osteoporosis: 4 – 5 y

Phase III

CONTROL group usually required based on the aim of the study:

- Absolute efficacy
- Comparative evaluation
- Risk/benefit
- Safety

Phase III

4 types of control:

- Versus placebo
- Versus no treatment
- Dose-response
- Active Control

Phase III

Placebo / no treatment:

Advantages:

it allows to demontrate the absolute efficacy and safety; shows the maximum of efficacy, reduction of the numenr of involved subjects, minimum of the systemic error coming form the expectancies of the patient and researcher.

Disadvantages:

ethycal problems, lack of comparison with existing therapies.

Phase III

Dose - response:

Patients are assigned casually to a certain dose, with or w/o placebo. The efficacy is based on the statistical comparisons between doses and with placebo.

Phase III

Active control:

- Drug already in the market
- obligatory
- Necessary at least to demonstrate the not-inferiority
- best tolerability
- To establish the price of the new drug

Phase IV

- post marketing study=pharmacovigilance
- Allow to evaluate the development of toxic effects at low frequency not detected during the phase II and III clinical trials.

Problems linked to the development of a new drug

- Lack of efficacy

- Toxic effects in :
  - Cellular models, animals, human

- Manufacturing problems:
  - impurities
  - Degradation products,
  - Unexpected metabolites during synthetic process

.....more

- Problems related to the pharmaceutic form:
  - stability,
  - Difficulties of formulation

- Mild or limited efficiay in a very competitive market

- Changes in the therapeutic approach to the pathology

Biotech drugs
Gene Therapy
Cell Therapy
Pharmacogenetics