



ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΕΡΓΑΣΤΗΡΙΟ ΒΙΟΜΑΘΗΜΑΤΙΚΩΝ

ΚΑΤΗΓΟΡΙΕΣ ΙΑΤΡΙΚΗΣ ΕΡΕΥΝΑΣ ΚΑΙ Η
ΠΥΡΑΜΙΔΑ ΤΩΝ ΕΝΔΕΙΞΕΩΝ
ΜΕΡΟΣ 2^ο

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Κατηγορίες Κλινικών Μελετών

- Παρεμβατικές (Interventional): ο ερευνητής επεμβαίνει στη θεραπεία.
- Μη Παρεμβατικές (Observational): παρατήρηση χωρίς παρέμβαση.
- Ειδικές μορφές: υβριδικές, registry, real-world evidence.



Παρεμβατική
Μελέτη



Μη Παρεμβατική
Μελέτη

Παρεμβατικές Μελέτες (Interventional)

- Randomized Controlled Trials (RCTs): gold standard.
- Χορήγηση φαρμάκου ή θεραπείας με έλεγχο και τυχαιοποίηση.
- Σκοπός: να αποδειχθεί αιτιώδης σχέση μεταξύ παρέμβασης και αποτελέσματος.

The Hierarchy of Evidence



Ομάδες Ελέγχου (Control Groups)

- Placebo control – σύγκριση με ανενεργό ουσία.
- Active control – σύγκριση με εγκεκριμένη θεραπεία.
- Historical control – χρήση δεδομένων παλαιότερων μελετών.

Μεροληψία και Παρεμβολές (Bias & Confounding)

- Selection bias, Performance bias, Detection bias.
- Confounding: τρίτος παράγοντας επηρεάζει τη σχέση έκθεσης-αποτελέσματος.
- Χρήση στατιστικής προσαρμογής ή σχεδιασμού για μείωση bias.

Μηχανισμοί Μείωσης Μεροληψίας

Τυχαιοποίηση (Randomization)

Τυφλοποίηση (Blinding)

Απόκρυψη Κατανομής (Allocation Concealment)

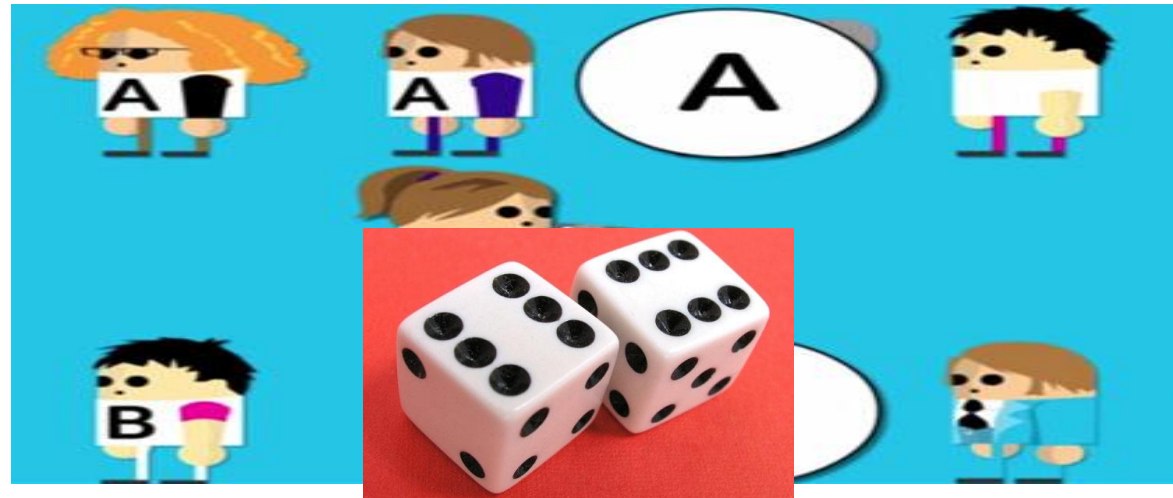
Τυποποίηση Διαδικασιών (Standardization)

Χρήση Αντικειμενικών Endpoints

Randomization (Τυχαιοποίηση)

Ισότιμη κατανομή χωρίς προκατάληψη

- *Individuals are allocated at random to receive one of several interventions (at least two at total)*



- *gold standard of EBM*

Randomization Allocation

- Covariates are distributed equally across the groups at baseline
- Affects both measured and unmeasured variables

Note: the risk of the imbalance remains even after properly executed randomization

Generation of allocation sequence

- ***Simple Randomization***

- *Analogous to a repeated fair coin tossing*

- ***Restricted randomization - Blocking***

- *Done to ensure equal balance of arms throughout all portions of the study*

- *i.e. blocks of six would have 3 active/3 control*

- ***Stratified randomization***

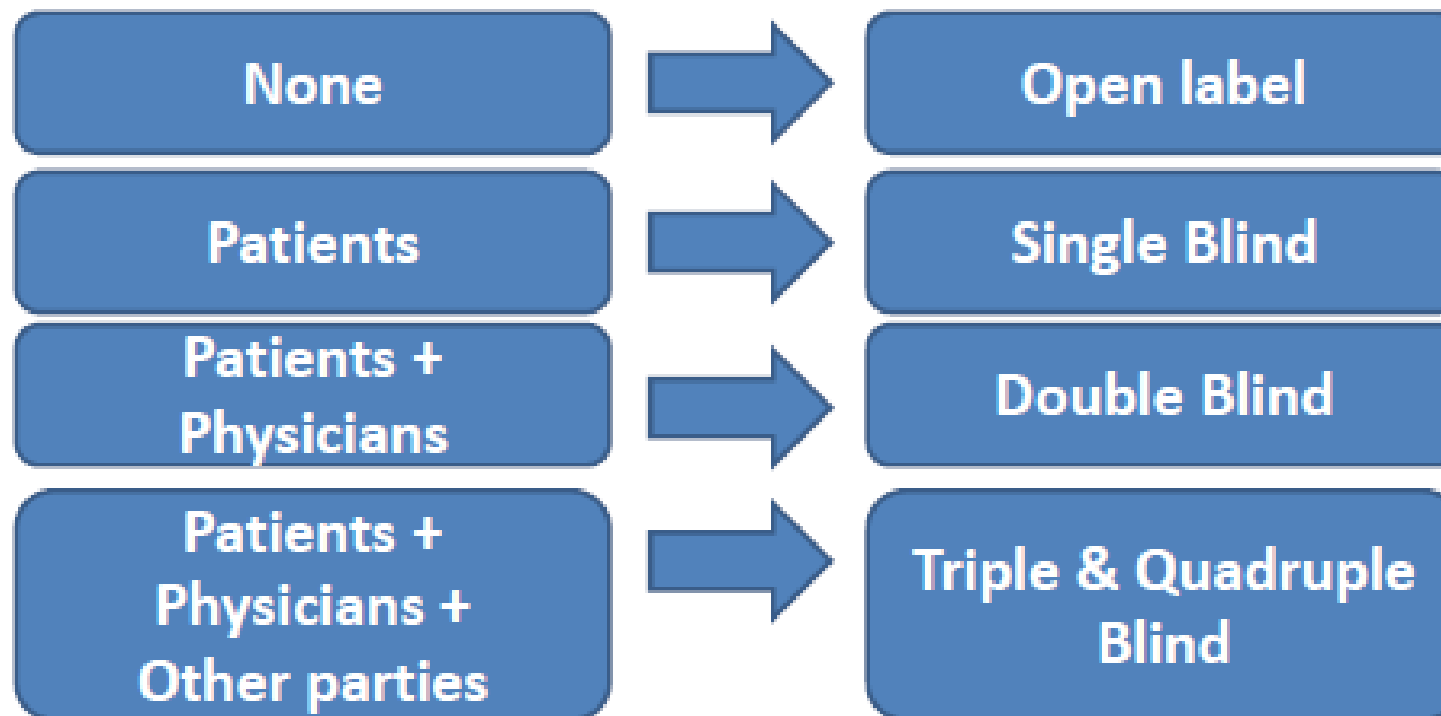
- *Individuals are identified based on important covariates (sex, age, etc.) and then randomization occurs within the strata*

Τυφλοποίηση (Blinding)

- Αποτρέπει την επιρροή υποκειμενικών παραγόντων.
- Single-blind, double-blind, open-label.
- Η αποκάλυψη (unblinding) γίνεται μόνο για λόγους ασφάλειας.

Blinding

- Classification based on whether investigators and/or participants know which intervention is being studied



Classification Schemes for RCTs

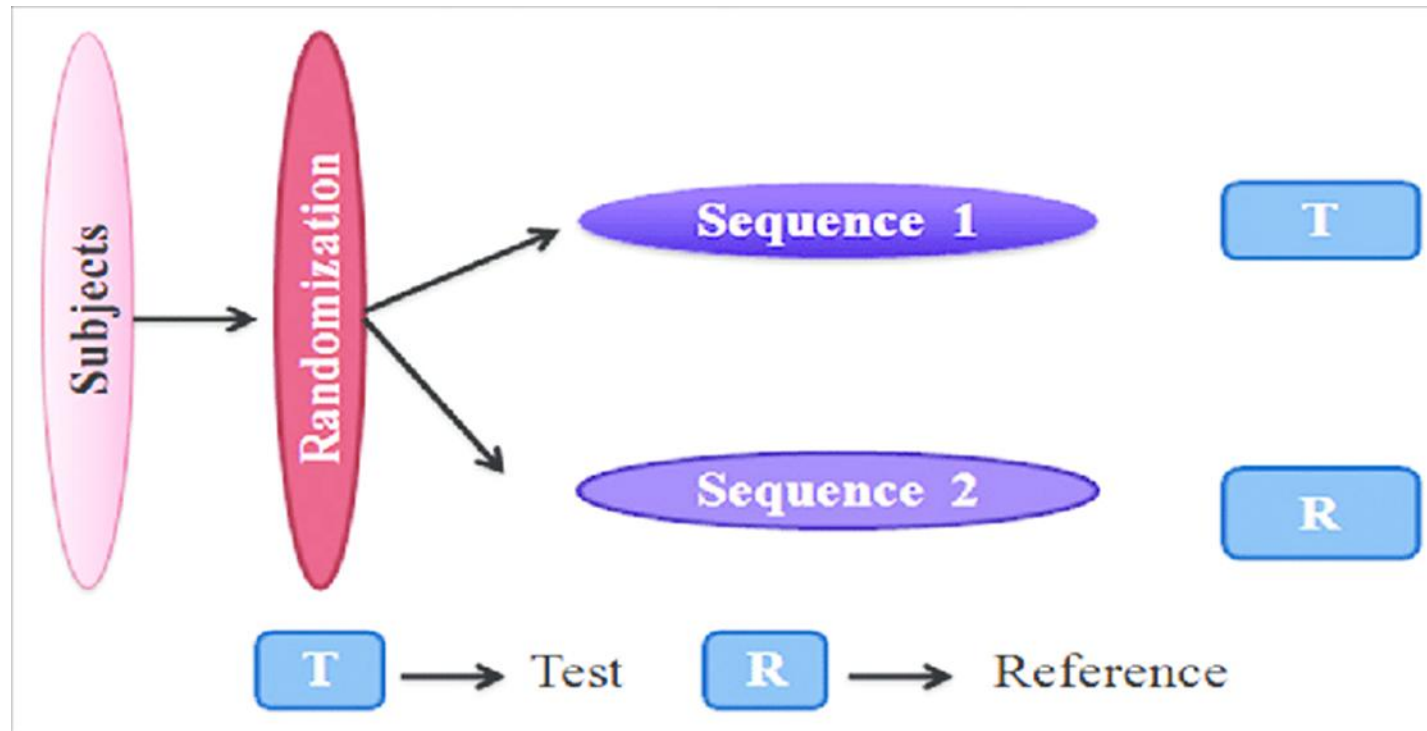
- Based on whether investigators and/or participants know which **intervention is being studied (BLINDING)**
- Based on **how participants are exposed** to interventions
- Based on **the type of interventions** being evaluated
- Based on **the number of participants**
- Based on whether **the goal** is evaluation of superiority, equivalence or non-inferiority

Classification based on how participants are exposed to interventions

- Parallel Trials
- Crossover Trials
- Trials with Factorial Design

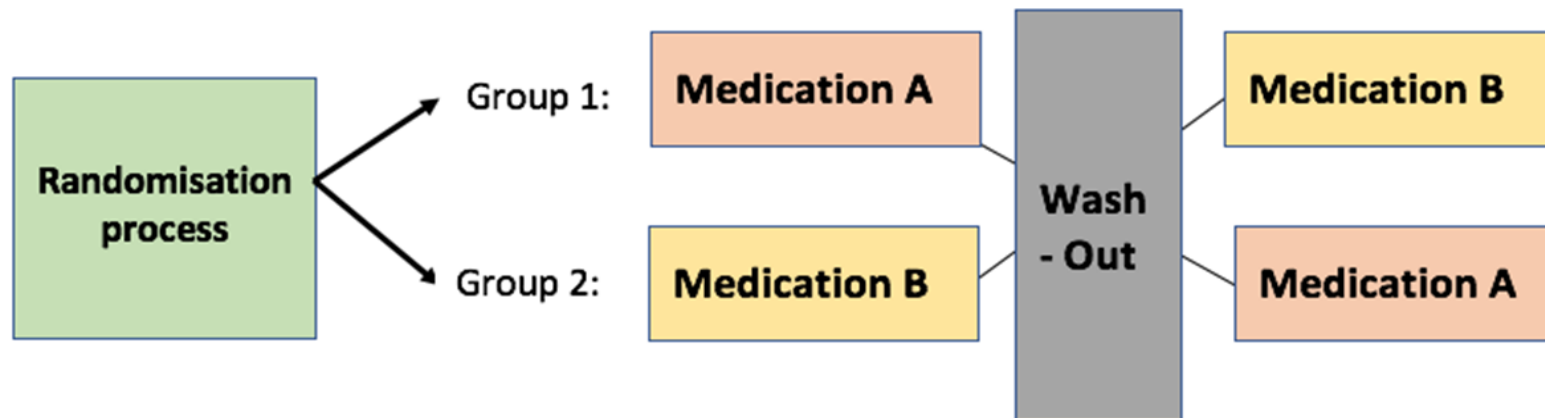
Parallel RCT design

- the most commonly used study design
- subjects are randomized to one or more study arms
- each participant will stay in their assigned treatment arm for the duration of the study



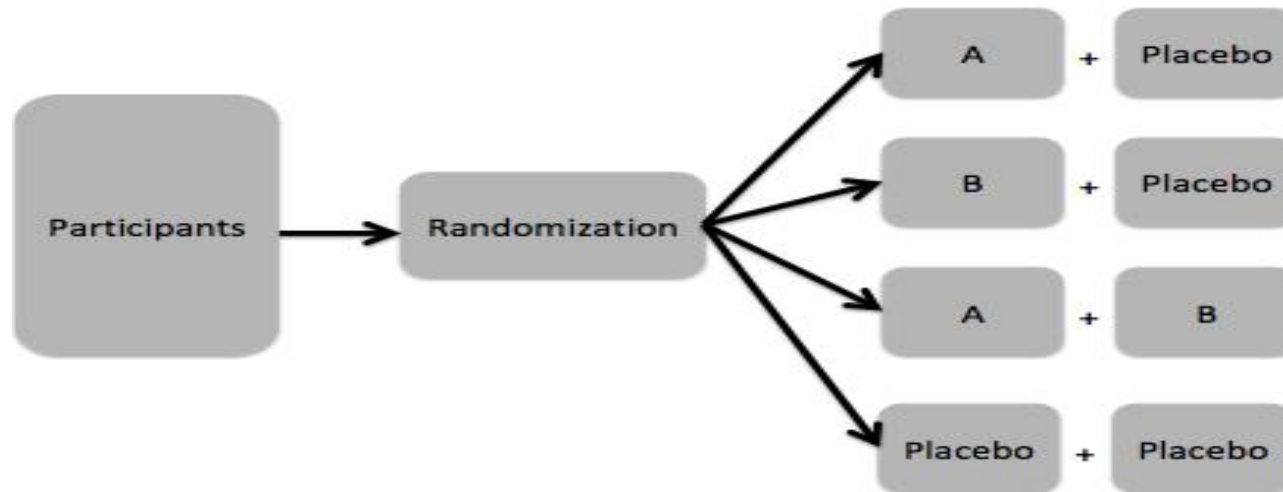
Crossover RCT design

- each subject receives both interventions but in a different order.
- The order in which subject receives interventions is randomized.
- Each subject serves as his/her own control results in balancing the covariates in treatment and control arms.
- Adequate washout period must be given before crossover to eliminate the effects of the initially administered intervention.
- Bioequivalence and biosimilar equivalence studies usually utilize a cross over design.

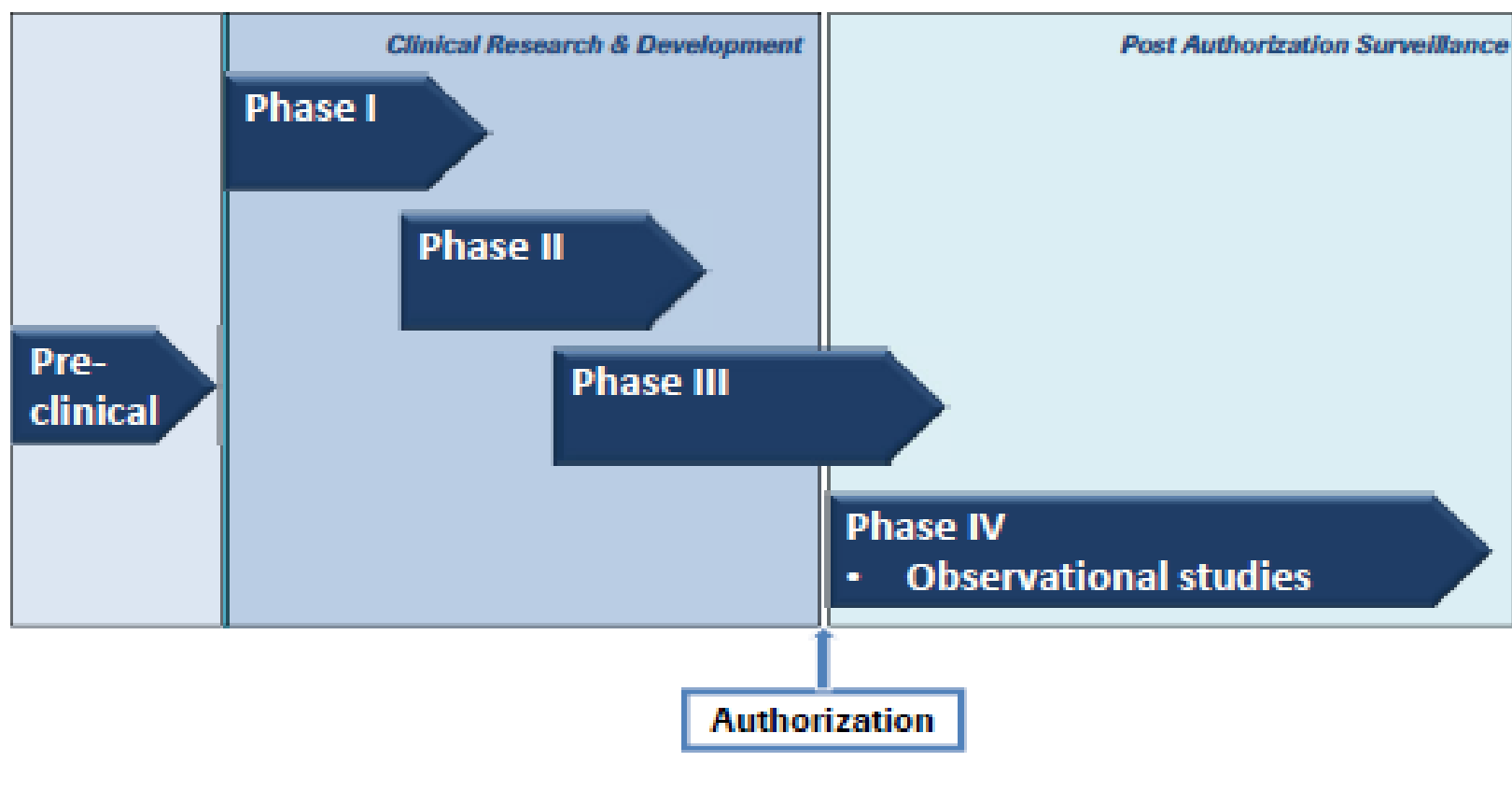


Factorial clinical trial

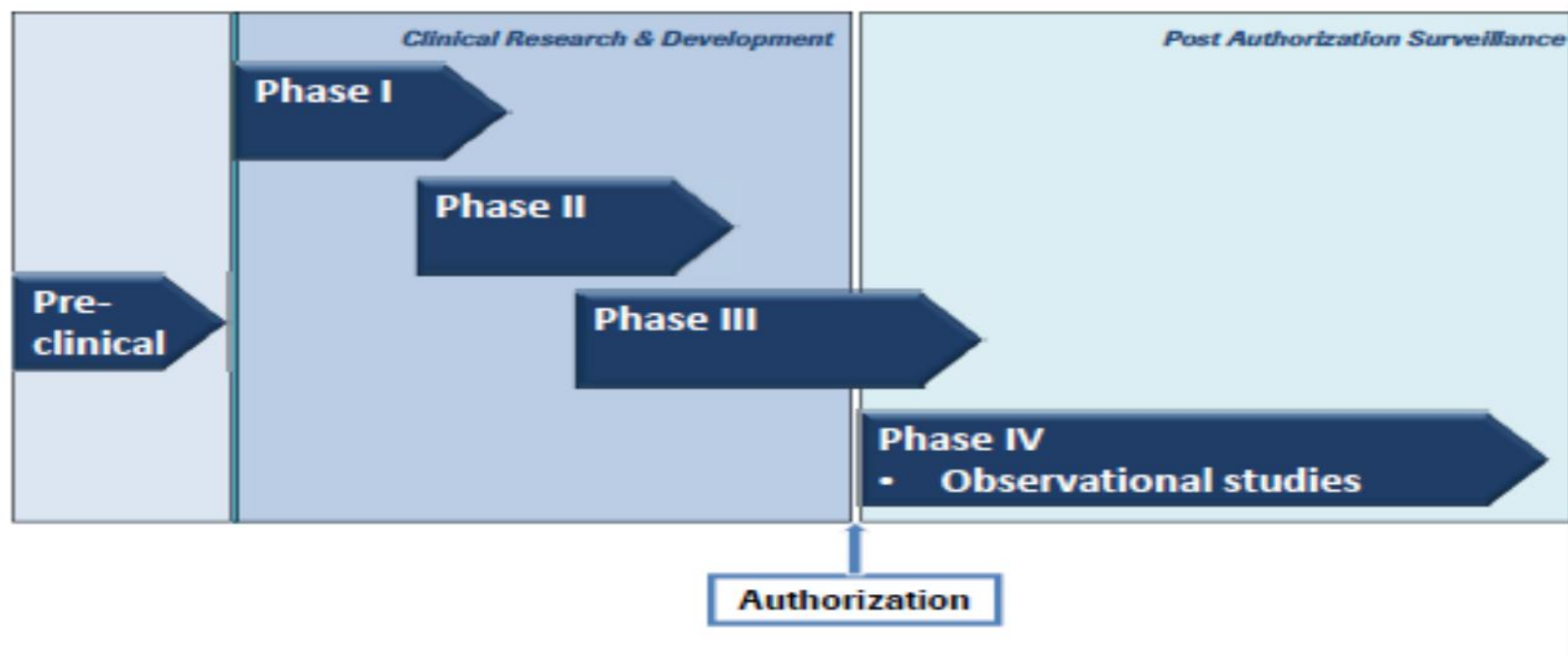
- the effect of two or more treatments simultaneously
- various combinations of the treatments.
- The simplest factorial design is known as a 2x2 factorial design, → four combinations of two interventions.



Drug development phases

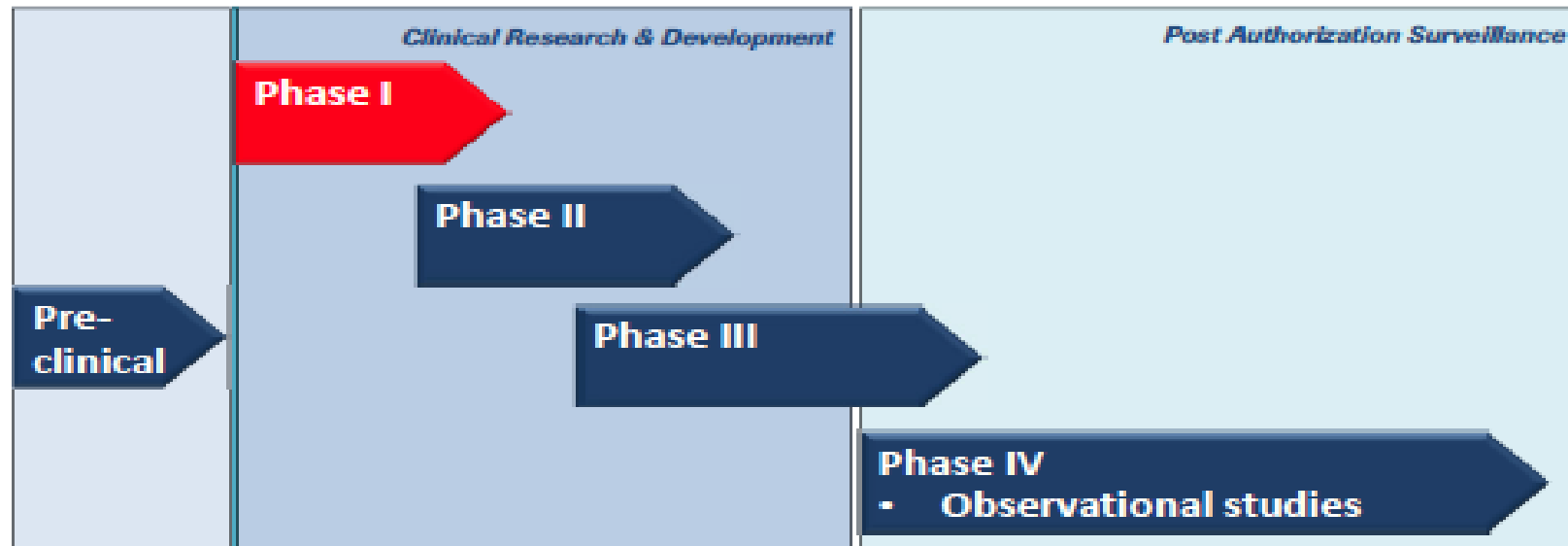


Phase 0 (Exploratory IND series)



- the first clinical trials done among people.
- Aim: how a drug is processed in the body and how it affects the body.
- a very small dose of a drug is given to about 10 to 15 people.

Phase I



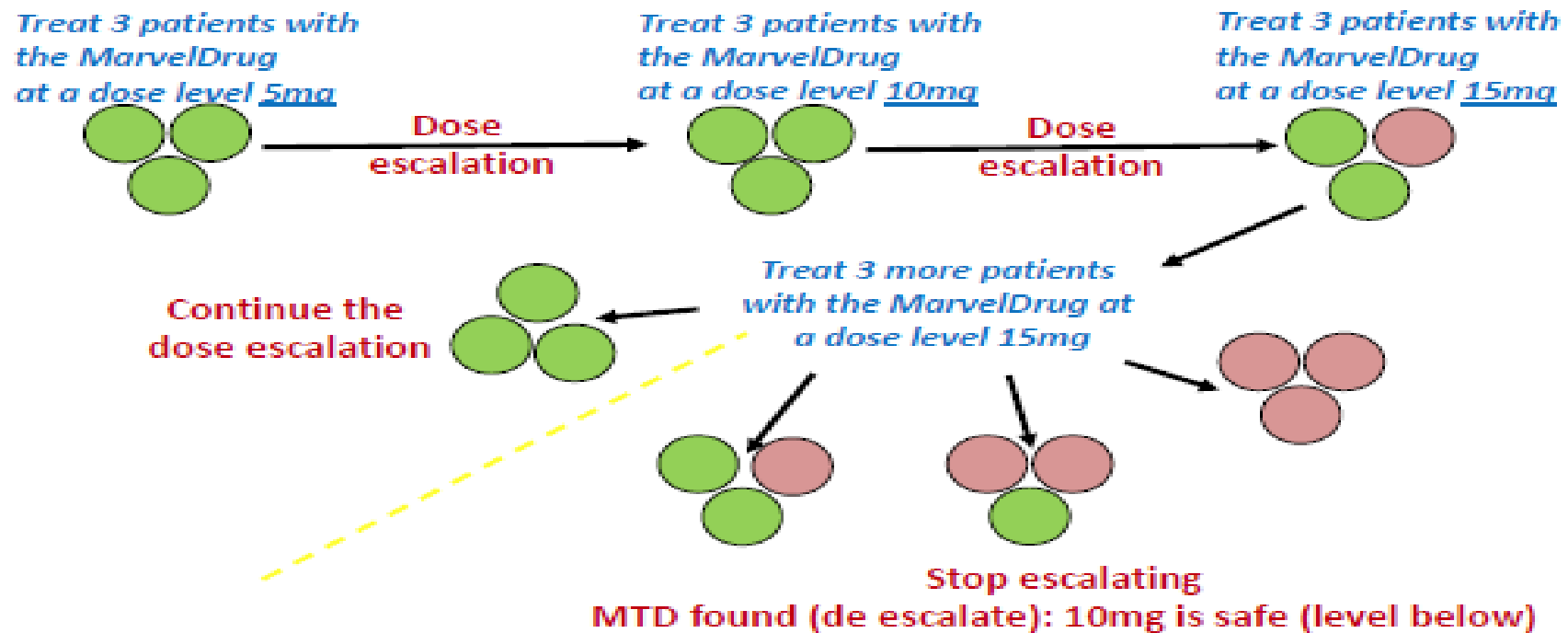
- **Aim:** drug's safety → human tolerance- dose range
- Pharmacokinetics/-dynamics.
- Administered form (pill, solution, patch)
- small group of 15 to 30 patients (<100).



< 100



≤ 1 year

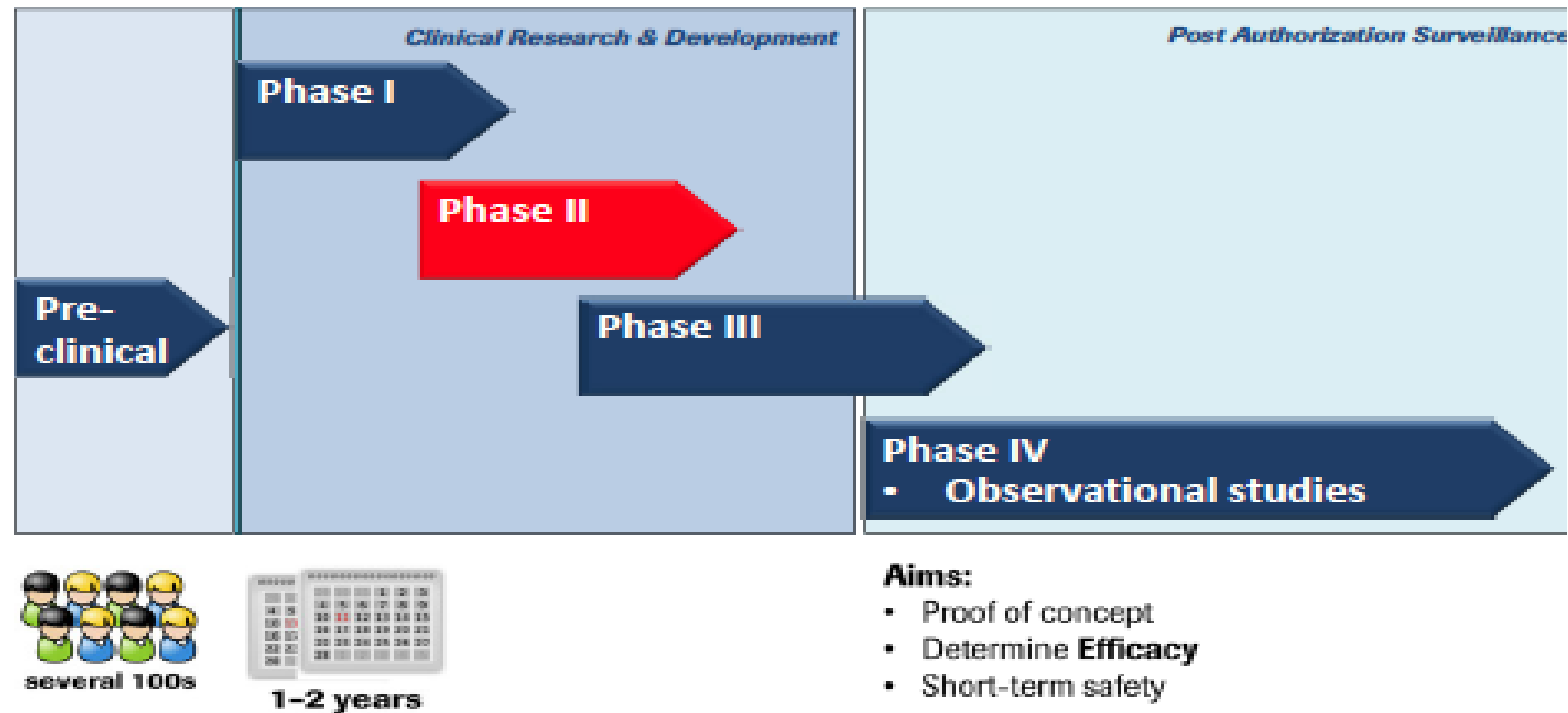


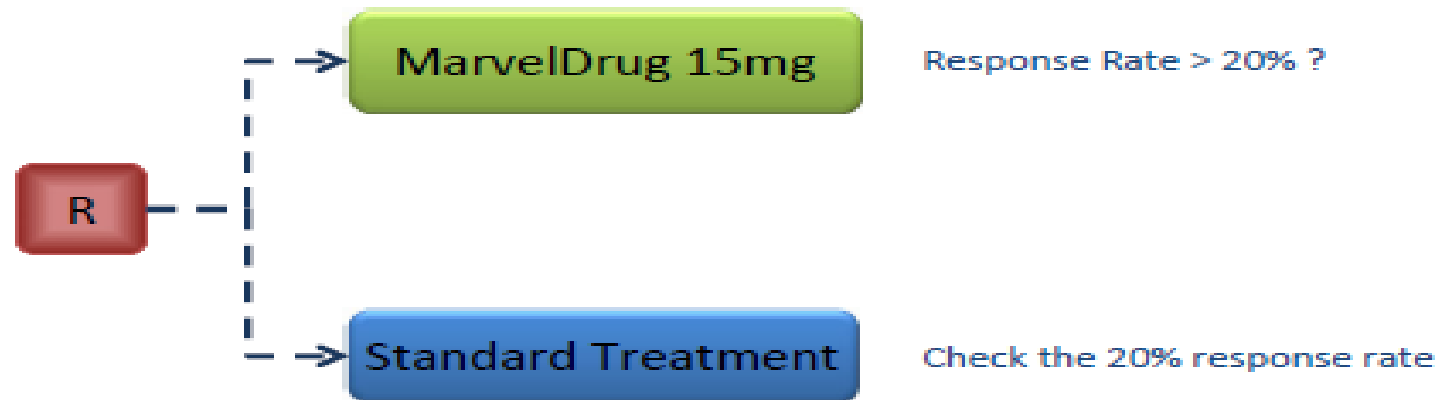
DLT: Dose Limiting Toxicity
MTD: Maximum Tolerated Dose



Phase II

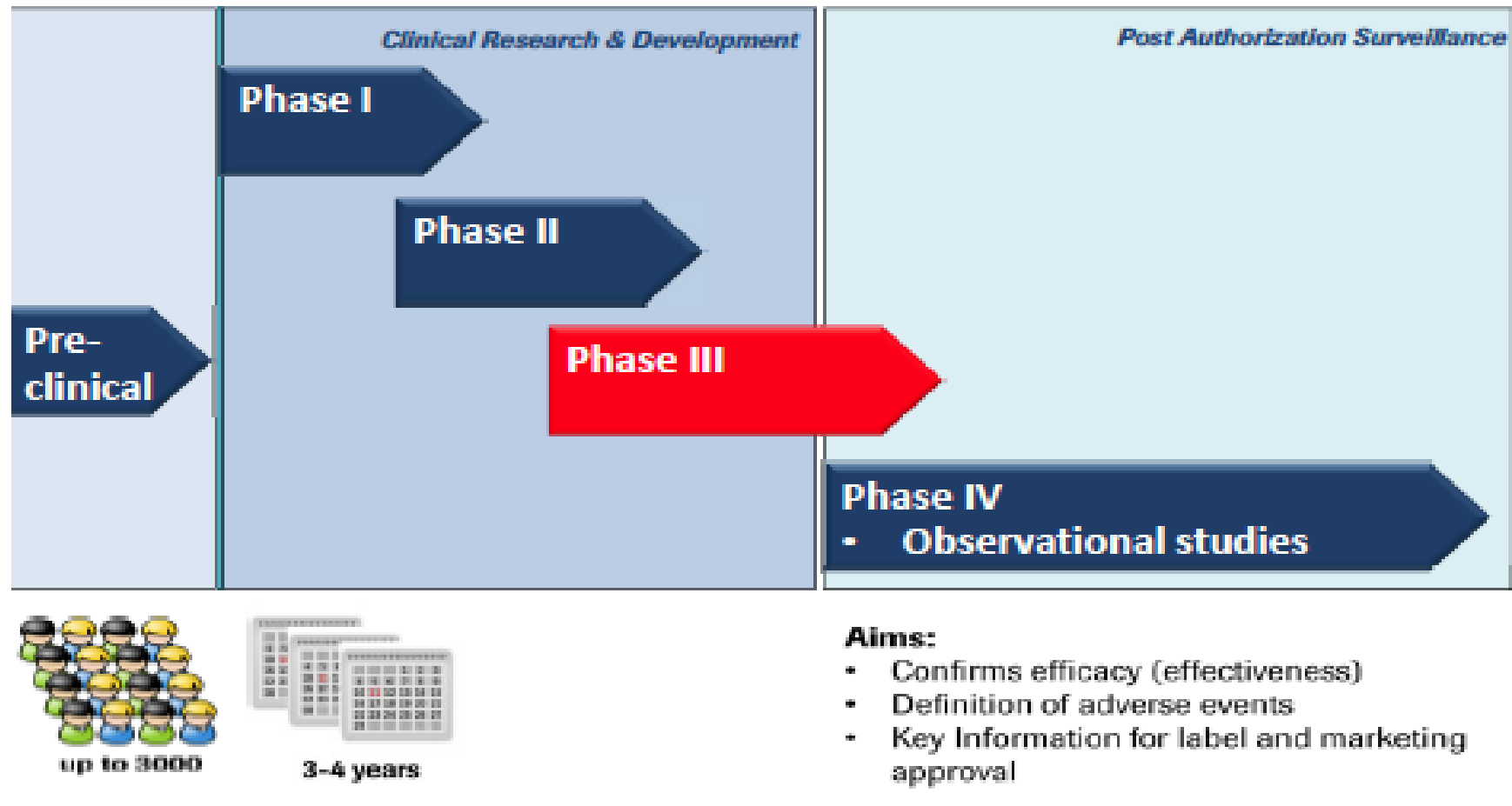
Phase II

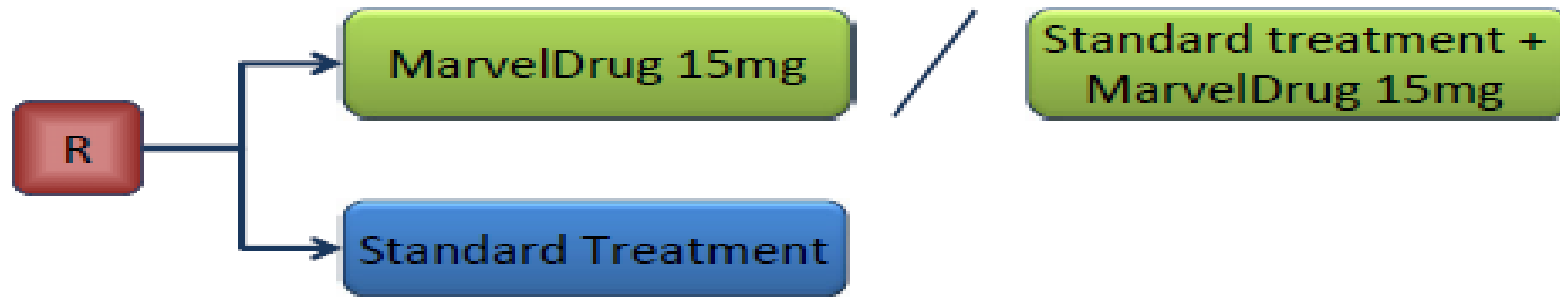




Objective: to show that more than 20% of the patient treated with the MarvelDrug will respond
(i.e. a response rate $\leq 20\%$ would suggest inactivity, usually level of activity is determined on clinical grounds)

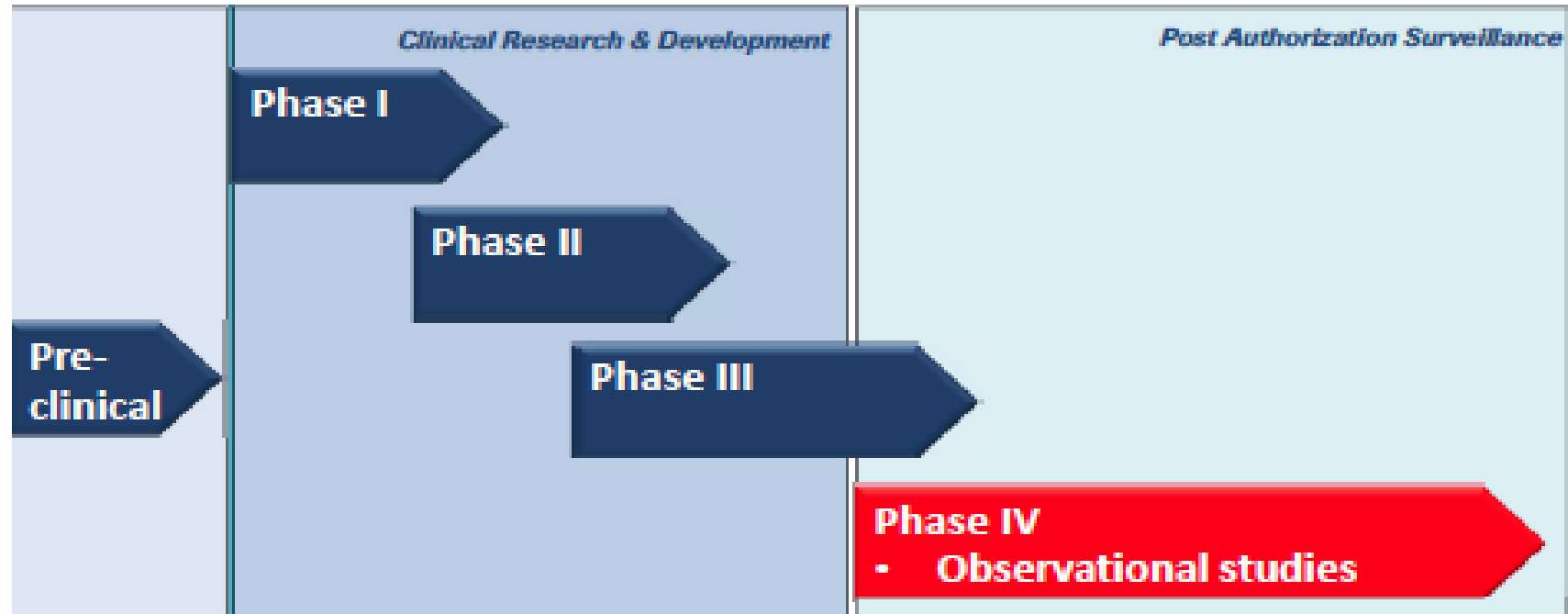
Phase III





Superiority Trial / Equivalence / Non-Inferiority

Phase IV



Up to
10000

Aims:

- New formulations and indications
- Long - term safety
- Identification of rare adverse events in comparison to other standard treatment

Superiority trials

Purpose

- To detect a difference between two drugs

Goals

- Establish new drug is statistically superior to active control (and/or placebo)
- Establish new drug is clinically superior to active control (and/or placebo)

Equivalence Trials

Purpose

- To confirm the absence of a meaningful difference between treatments

Bioequivalence Studies (BE)

- **Bioavailability:** Bioavailability is the rate and extent to which the active ingredient or active moiety is absorbed from a drug/product and becomes available at the site of action (USFDA)
- **Bioequivalence** (comparative bioavailability): the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study

Bioequivalence Studies (BE)

- **Bioavailability:**
- Bioavailability data provide an estimation of the fraction of the drug absorption, distribution, metabolism, excretion, and the effects of food on the absorption of the drug, dose proportionality, or linearity in the pharmacokinetics of the active moieties and when appropriate inactive moieties.

Bioequivalence Studies (BE)

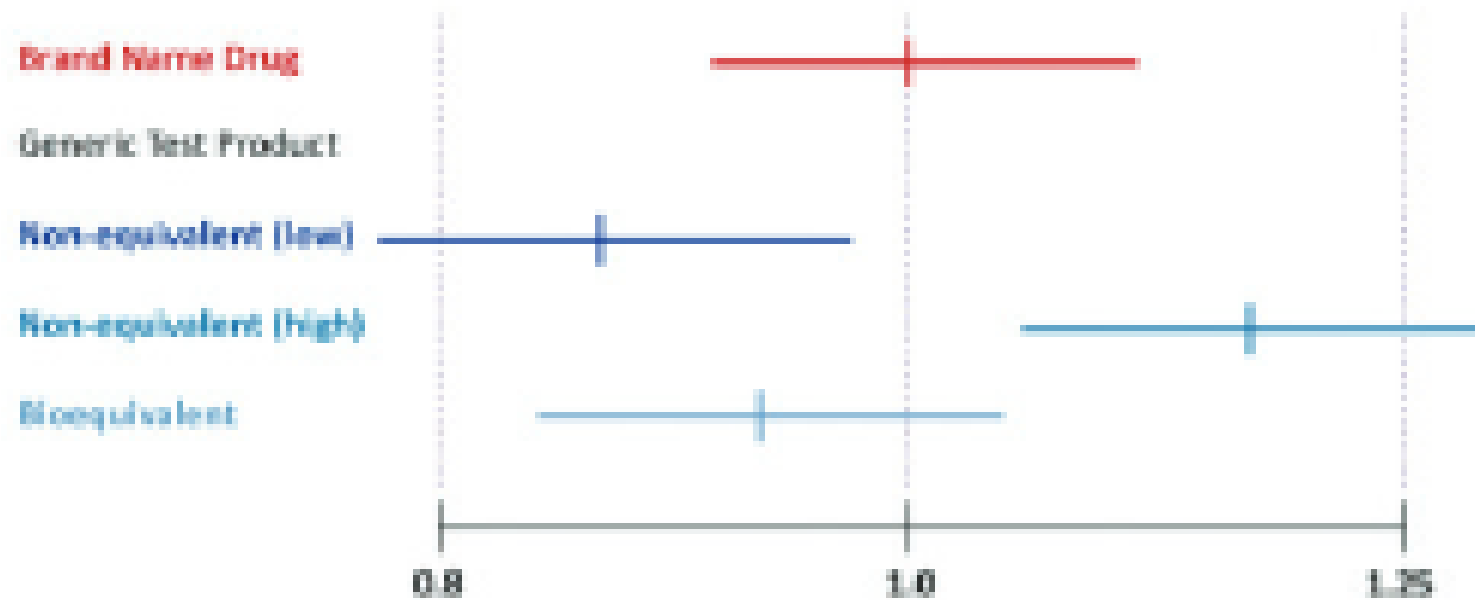
- **Bioequivalence** (comparative bioavailability): measure and compare formulation performance between two or more drug products.
- Bioequivalence is measured based on the relative bioavailability of the innovator drug product versus the generic drug/product.
- BE studies with Pharmacokinetic endpoint ≠

Bioequivalence Studies (BE)

- BE studies are also performed for innovator drug product in some situations such as:
- between early and late clinical trial formulations;
- between formulations used in clinical trials and stability studies, if different;
- between clinical trial formulations and to-be-marketed drug products, if different;
- between equivalence of product strengths;
- between marketed innovator product and a new formulation of marketed innovator product (change in one or more excipients).

Figure 2: Testing for Bioequivalence

Criteria for Acceptance: 90% confidence interval of the ratios of AUC falls between 0.80 and 1.25 of the branded drug.



Non-Inferiority (NI) Trials

- Purpose:

To demonstrate that a new drug is not worse than an active comparator by more than a pre-specified amount*

- Non inferiority margin delta (Δ), δ
- NOT equivalent
- NOT inferior to active comparator
- NI margin must be determined by combination of clinical considerations and statistical methods

- Ο σχεδιασμός καθορίζει την εγκυρότητα και αξιοπιστία μιας μελέτης.
- Κάθε μελέτη απαντά σε ένα σαφές ερώτημα.
- Δύο βασικές κατηγορίες: Παρεμβατικές και Μη Παρεμβατικές.

Μη Παρεμβατικές Μελέτες (Observational)

ΜΟΝΟ ΠΑΡΑΚΟΛΟΥΘΗΣΗ

- Cohort studies – prospective/retrospective.
- Case-control studies – σύγκριση με βάση την έκθεση.
- Cross-sectional studies – αποτύπωση μιας χρονικής στιγμής.

Μελέτες Παρατήρησης

- Μελέτες Κοορτής

(Cohort Studies)

- ✓ ομάδα ατόμων με κοινό χαρακτηριστικό
- ✓ μια καλά σχεδιασμένη μελέτη κοόρτης μπορεί να παράσχει σημαντικά και αξιόπιστα αποτελέσματα
- ✓ Προοπτική μελέτη: πληροφορίες από τη στιγμή της έναρξης της μελέτης και έπειτα (συνήθως για μεγάλο χρονικό διάστημα)



- **Cohors**: subdivision of Roman Legion (300-600 legionnaire)
- **Group of individuals with common characteristics**

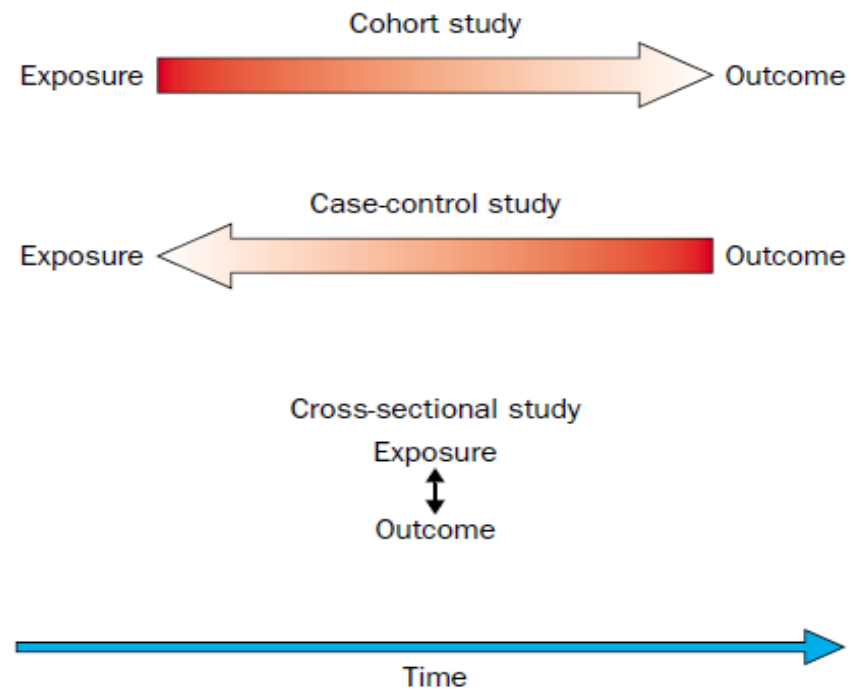
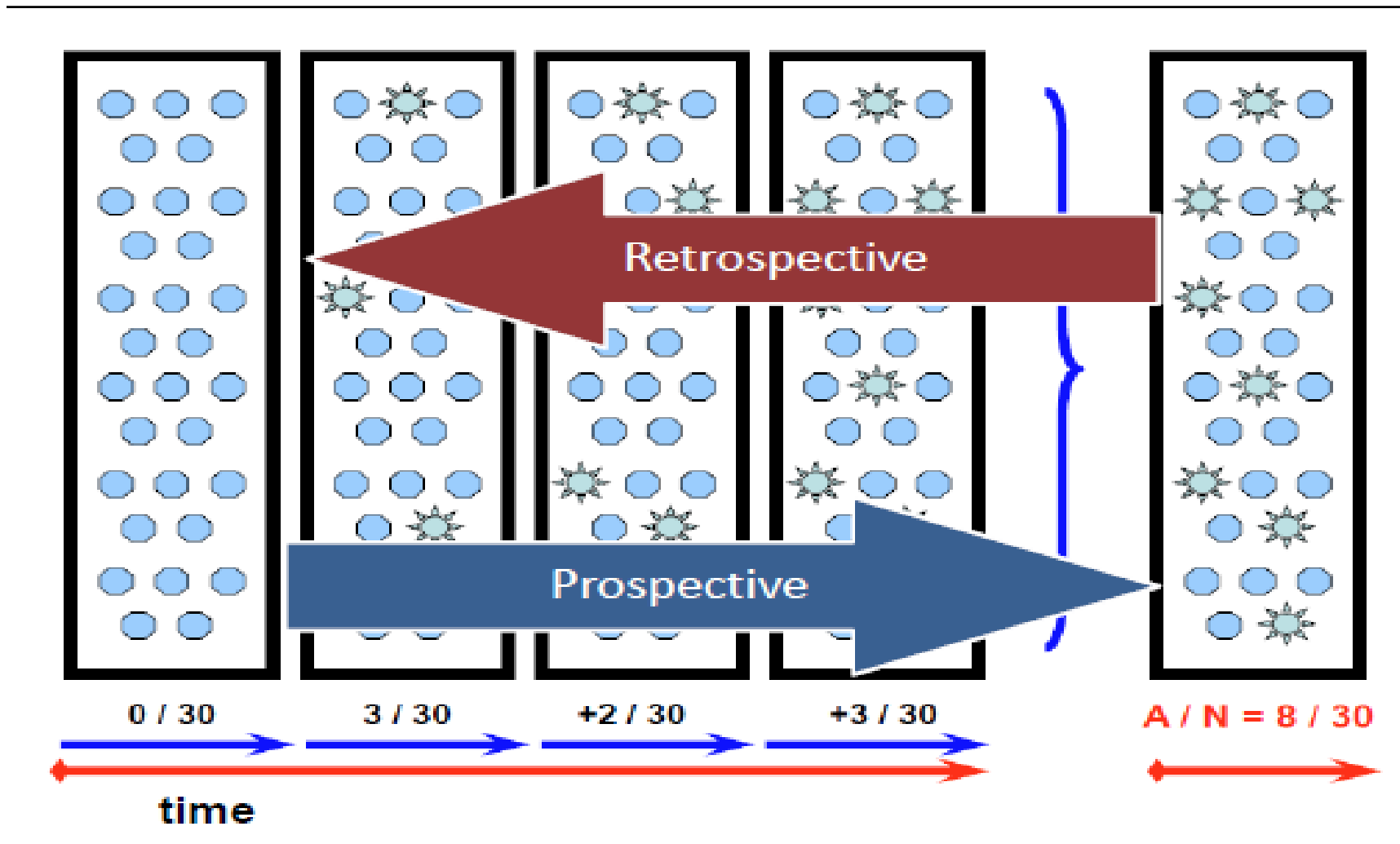


Figure 2: **Schematic diagram showing temporal direction of three study designs**





- Give direct information on the sequence of happenings (ideal for demonstrating causality)
- Permit the direct calculation of incidence rates in both the exposed and unexposed groups (calculation of risk or rate ratios, or differences)
- Permit **multiple outcomes** to be assessed in the same study
- Can be used to study exposures that are relatively uncommon (enable an adequate number of exposed and unexposed subjects at the study outset)



- **Expensive and time-consuming** (large sample size requirements and long follow-up periods)
- Withdrawals and losses to follow-up (**selection bias**) (connected or unconnected to the disease)
- **Exposure misclassification** (measurement bias). Subjects may change their exposure status during the follow-up period. Periodic reassessments of exposure status are necessary
- **Outcome misclassification**. Advances in the ability to detect a particular disease during the course of follow-up may bring prior classifications of outcome status into question and lead to incorrect study results
- Diagnostic suspicion bias. Knowledge of a subject's exposure status may influence the accuracy with which outcome status is determined
- Inefficient for rare diseases

Μελέτες Παρατήρησης

- Ασθενών-μαρτύρων

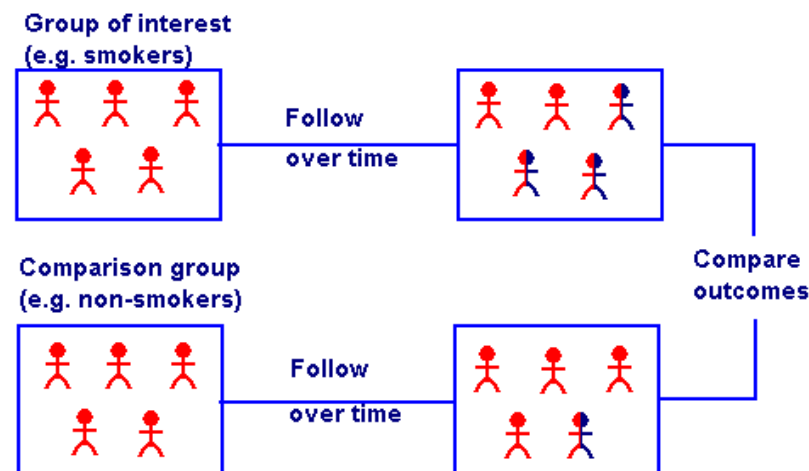
(Case-control studies)

Δύο ομάδες (two cohorts)

α) η ομάδα που εκτίθεται (case)

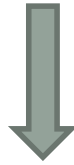
β) Η ομάδα σύγκρισης που δεν έχει τη νόσο (control) ή που είναι μη εκτεθειμένα αλλά παρόμοια κατά τα υπόλοιπα με την κοορτή των εκτεθέντων

πρέπει να επιλέγεται με τέτοιο τρόπο που, ως προς τους άλλους παράγοντες κινδύνου της υπό μελέτην νόσου, να είναι όμοια με την ομάδα των εκτεθέντων.



συγχρονικές μελέτες (cross-sectional studies)

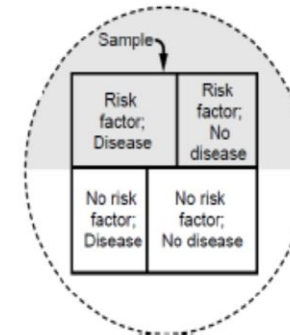
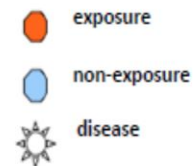
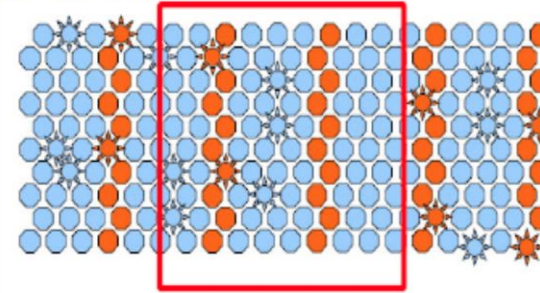
Παρατήρηση σε μια συγκεκριμένη χρονική στιγμή



1 η στιγμή της μέτρησης για κάθε συμμετέχοντα
πολύ μεγαλύτερο συστηματικό σφάλμα σε σχέση με
τις αντίστοιχες μελέτες κοόρτης και μελέτες

«ασθενών- μαρτύρων»

cross-sectional



Risk factor; Disease	Risk factor; No disease
No risk factor; Disease	No risk factor; No disease

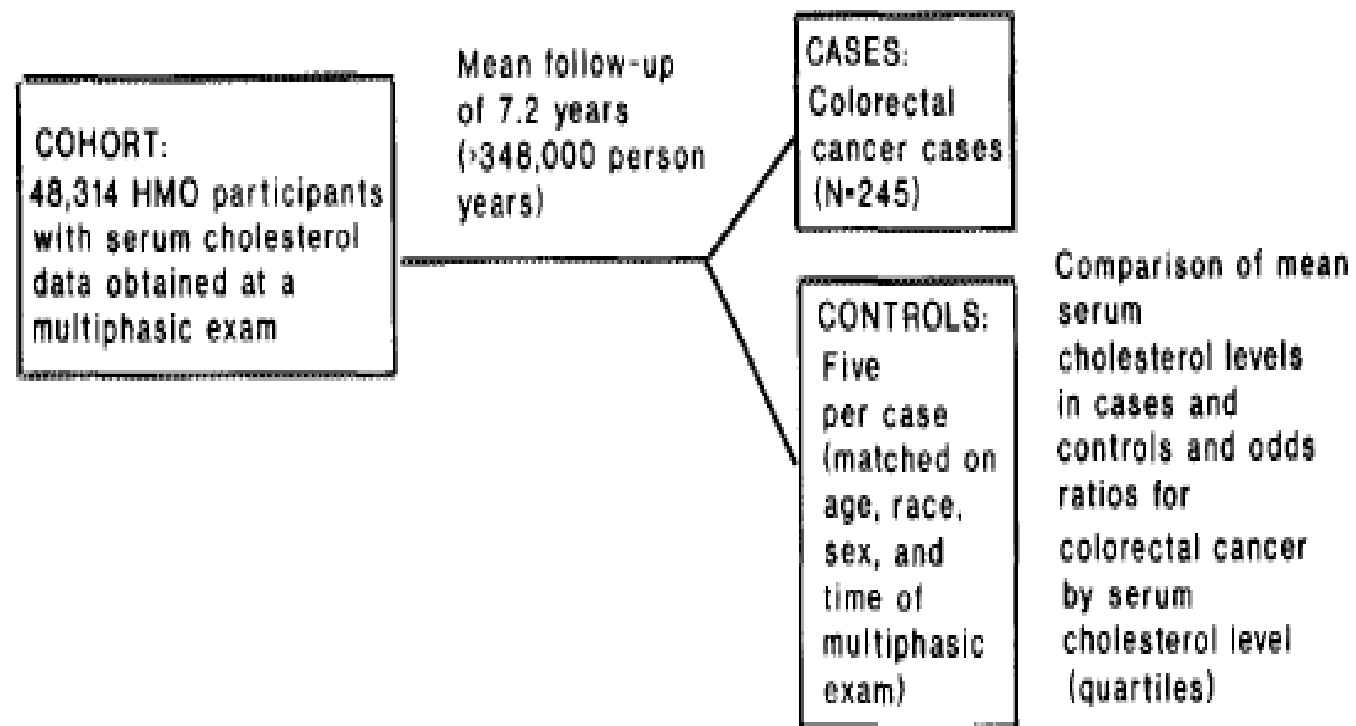
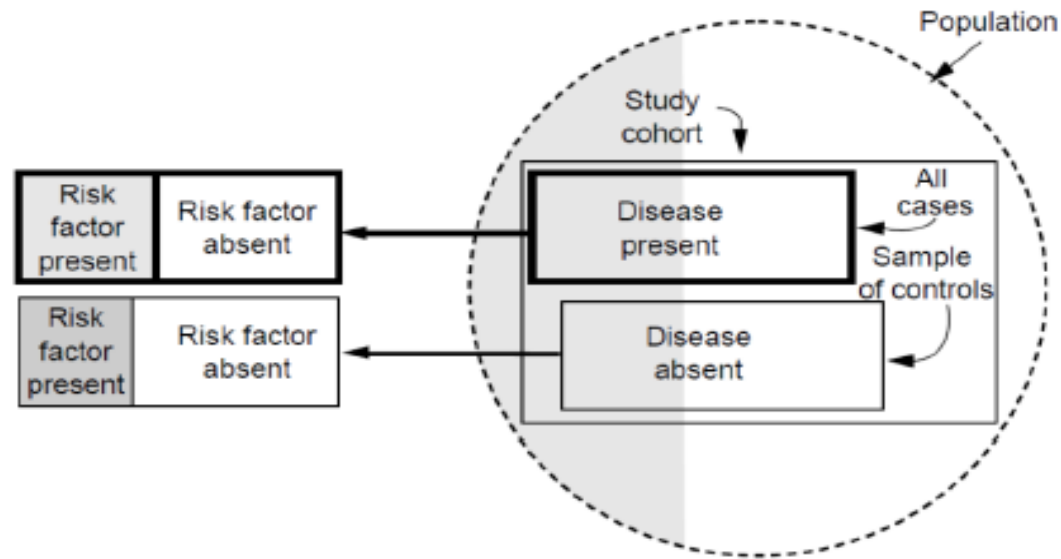


FIG. 1. Example of a nested case-control study (11) on serum cholesterol and large bowel cancer.

Nested Case-Control / Case-Cohort Studies

MEASUREMENTS IN THE PRESENT OF
SPECIMENS FROM THE PAST

THE PRESENT



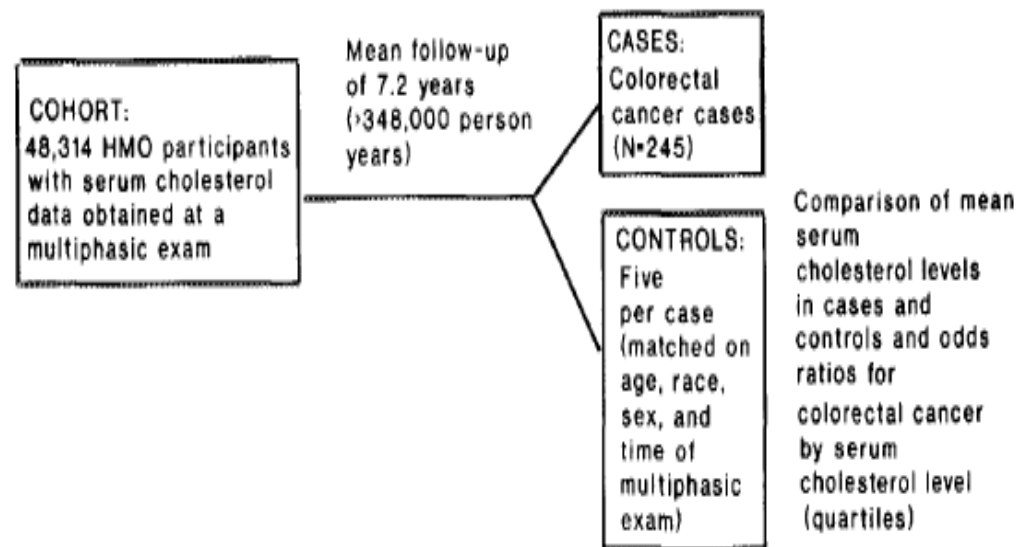


FIG. 1. Example of a nested case-control study (11) on serum cholesterol and large bowel cancer.

Case report/ Case series

- ✓ περιγραφές περιπτώσεων χωρίς τη χρήση ομάδας ελέγχου
- ✓ χρήσιμη για τη δημιουργία υποθέσεων
- ✓ Βάση για τον σχεδιασμό αναλυτικών μελετών

Μελέτες Παρατήρησης

Βήματα

- Καθορισμός ερωτήματος
- Επιλογή πληθυσμού μελέτης
- Μέτρηση της έκθεσης
- Παρακολούθηση
- Επιβεβαίωση της έκθεσης
- Ανάλυση των δεδομένων
- Ερμηνεία αποτελεσμάτων

TABLE 1 Types of clinical studies with related study designs and examples

Types of clinical studies	Study designs	Studied association	Examples
Analytic			
Experimental	Randomized controlled trial	Between an intervention and an intended outcome	What is the effect of benazepril plus amlodipine vs benazepril plus hydrochlorothiazide on the progression of CKD? ¹⁰
Observational analytic	Cohort study, case-control study, some cross-sectional studies	Between an intervention and an intended outcome	What is the difference in survival among ESRD patients on dialysis, on a transplant waiting list, and after receiving a renal transplant? ²⁷
		Between an exposure ³ and outcome	What is the risk of all-cause mortality in each stage of CKD? ¹⁷
		Between an intervention and unintended outcome	What are the effects of ACE/All inhibitors use vs no use, on peritoneal membrane transport characteristic in long-term PD patients? ²¹
Non-analytic			
Descriptive	Case reports, case series, cross-sectional studies (surveys)	Not applicable	What is the prevalence of CKD in individual European countries? ²⁵ What are the international time trends in the incidence of RRT for ESRD by PRD from 2005 to 2014? ²⁶

Abbreviations: ACE, angiotensin-converting enzyme; All, angiotensin-II; CKD, chronic kidney disease; CRF, chronic renal failure; ESRD, end-stage renal disease; PD, peritoneal dialysis; PRD, primary renal disease; RRT, renal replacement therapy.

^aHere exposure is defined as a naturally occurring exposure.

The Hierarchy of Evidence



Πλεονεκτήματα

- Διακρίνονται οι αιτίες από τις εκθέσεις. Η έκθεση μετριέται στην αρχή πριν την έκβαση.
- Παρέχονται πληροφορίες για τη φυσική ιστορία της νόσου.
- Μπορεί να υπολογιστεί ο σχετικός και απόλυτος κίνδυνος.
- Μπορεί ταυτόχρονα να μελετηθούν διάφορες εκβάσεις (αποτελέσματα)
- Ελαχιστοποίηση συστηματικών σφαλμάτων

Μειονεκτήματα

- Δεν μπορεί να επιβεβαιωθεί η αιτιολογική σχέση.
- Χρειάζονται μεγάλοι πληθυσμοί και συνήθως μεγάλοι χρόνοι παρακολούθησης (ανάλογα με το νόσημα υπό παρακολούθηση).
- Απαιτούνται περισσότεροι πόροι και αξιόπιστα αρχεία.
- Παρακολούθηση σε μεγάλο διάστημα-αποχωρήσεις.
- Δεν είναι κατάλληλη για σπάνιες νόσους.

Introduction to Genetic Epidemiology



DNA sequence of all human beings is 99.9% identical

⇒ Our DNAs differ by 0.1%.

⇒ Does it make a difference ?

Yes

0.1% difference translates into 3 million separate “spelling” differences in a genome of 3 billion bases

**Small variations in genotype can make
big differences to phenotype (1.24%)**



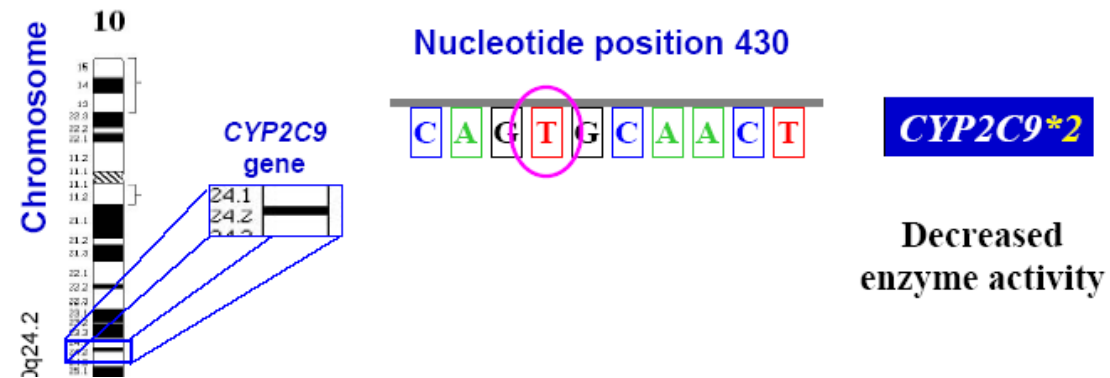
Single nucleotide polymorphisms (SNP)

.....G G **T** A A C T T G

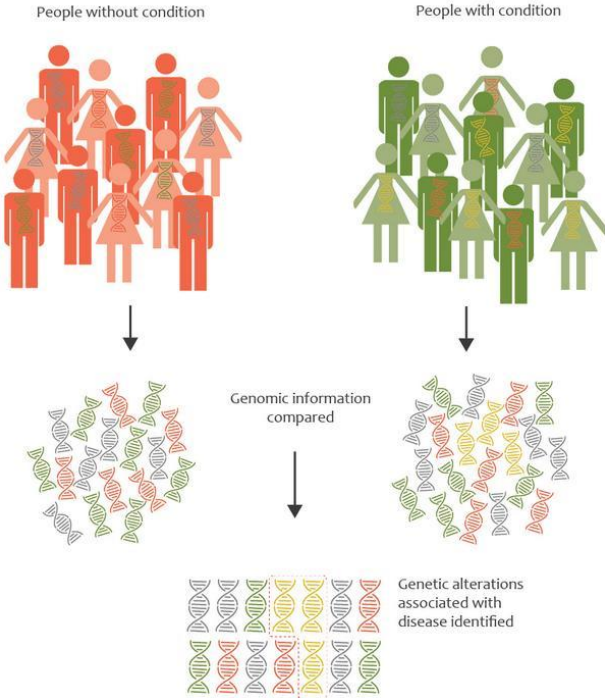
.....G G **C** A A C T T G

- Most common, Incidence 1 per 300 - 600bp

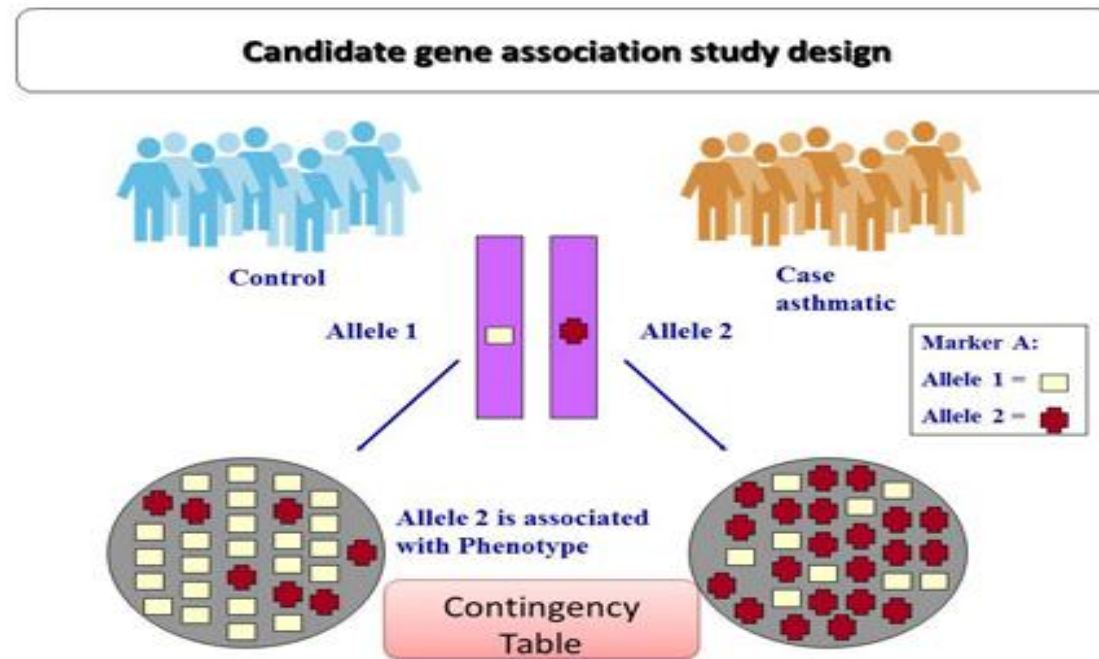
Example:



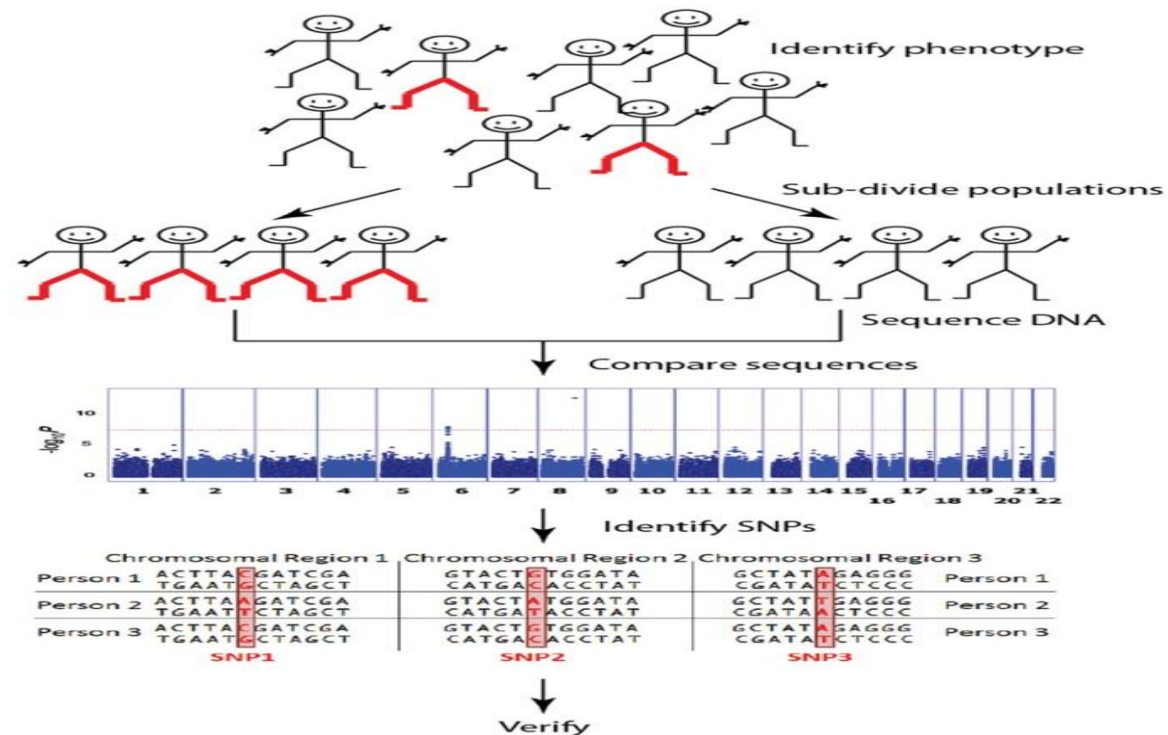
How researchers compare genomic information to identify genetic alterations

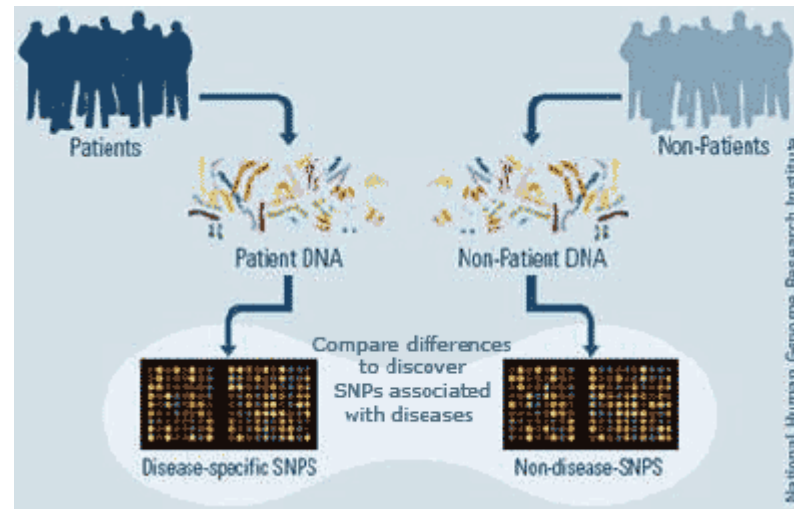


Genetic Association Studies (GAS)

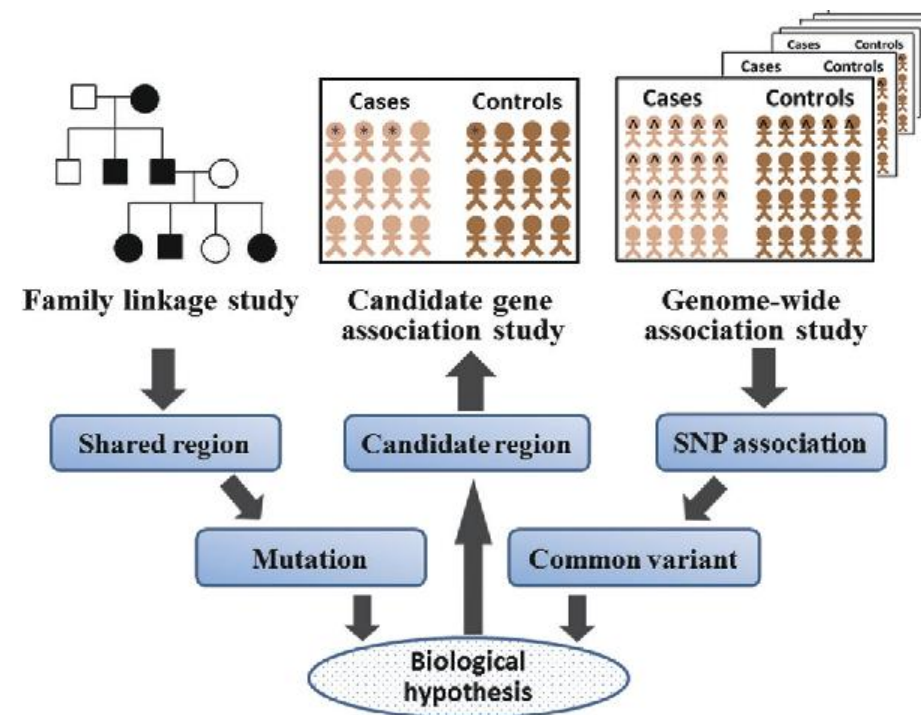


Genome-Wide Association Studies (GWAS)





Compilation of data from Genetic Epidemiology studies



Levels of Evidence Based Medicine

Level of Evidence	Qualifying Studies
I	High-quality, multicenter or single-center, randomized controlled trial with adequate power; or systematic review of these studies
II	Lesser quality, randomized controlled trial; prospective cohort study; or systematic review of these studies
III	Retrospective comparative study; case-control study; or systematic review of these studies
IV	Case-series
V	Expert opinion; case report or clinical example; or evidence based on physiology, bench research, or “first principles”

RCT vs Observational

- RCT → internal validity
- Observational → real-world
- Trade-off exists

Exam Question

- Which proves causality?
- A Cohort
- B Case-control
- C RCT (correct)
- D Cross-sectional

Take Home Messages

- Design > Results
- Bias is everywhere
- Think critically