Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

13 – BIOMARKERS

Summary and final notes

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Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

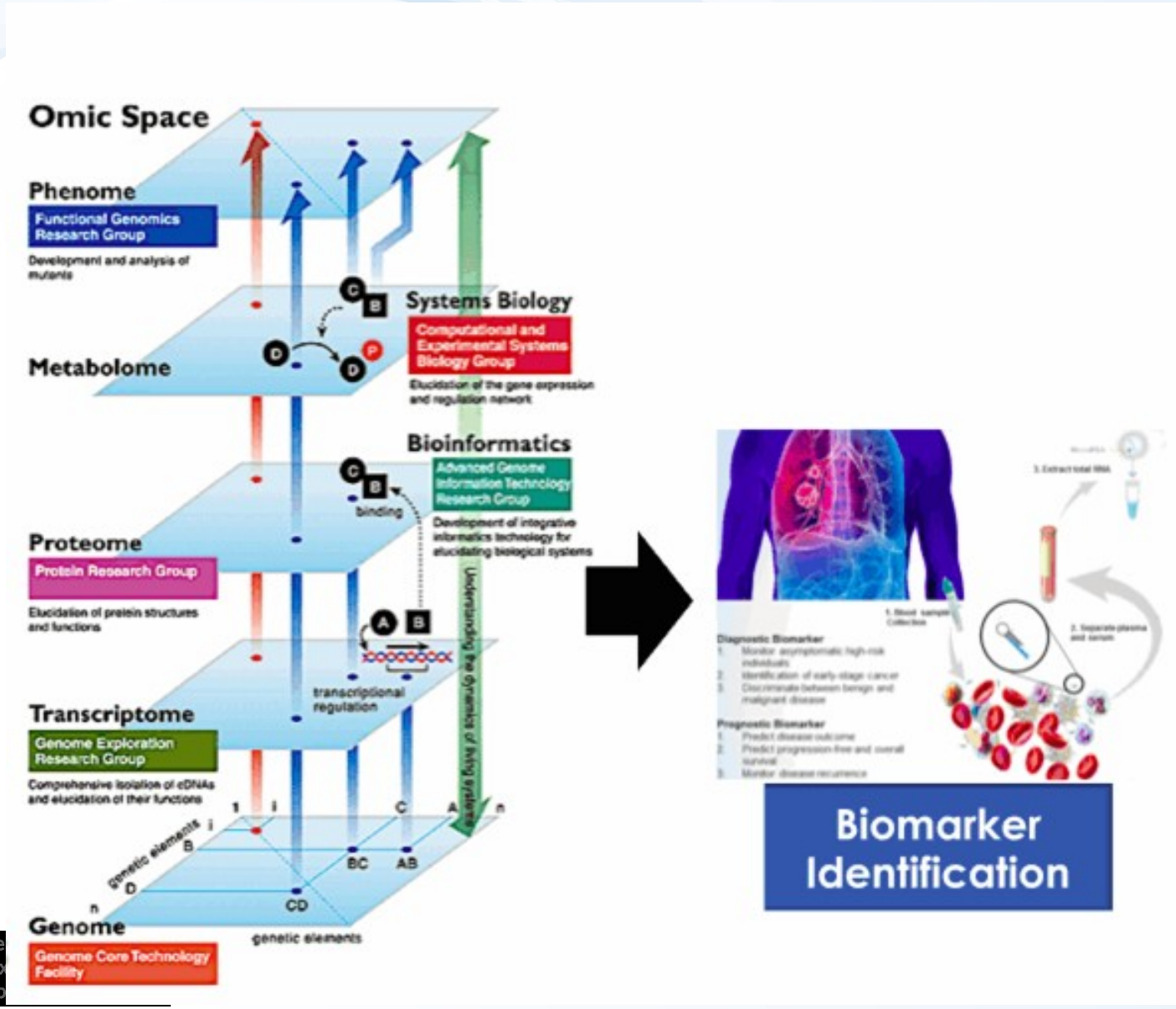
Topics covered in the final presentation

- Biomarkers at different levels
  - *Omics*
  - *... and beyond*

- Biomarkers in human medicine and drug development
  - Strategy and steps in development
  - Application examples

Biomarkers at various levels “omics”

Biomarkers at different biological levels – „omics“ approach



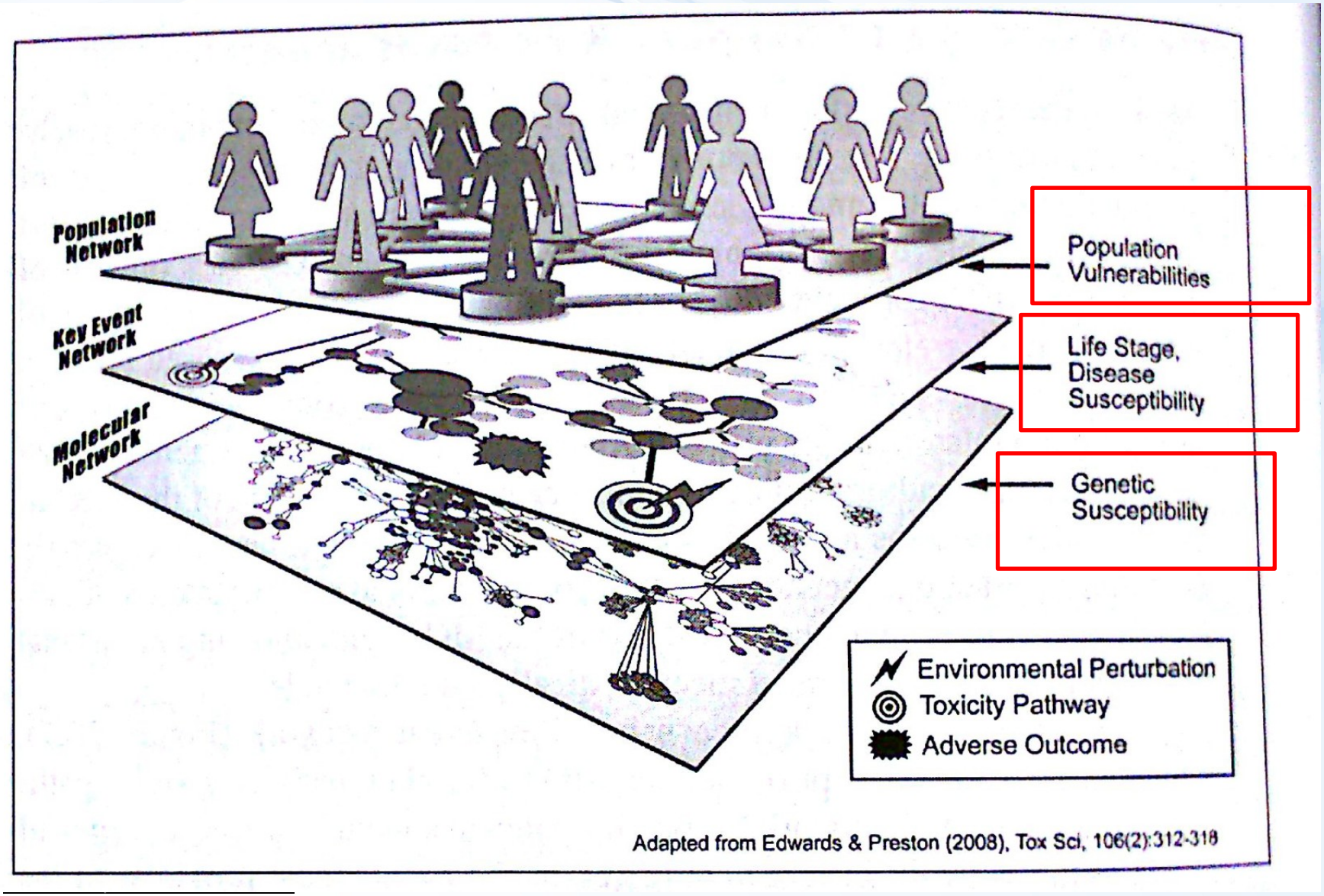
Biomarkers at different biological levels

- "Omics" techniques
  - Systems biology research
  - Screenings of responses (differences) at all levels of biological organization

- GENOMICS
  - Relatively stable
    - not responding to environmental changes (e.g. Toxicants)
  - Can be used as "biomarkers of susceptibility" (SNPs and personalized medicine)

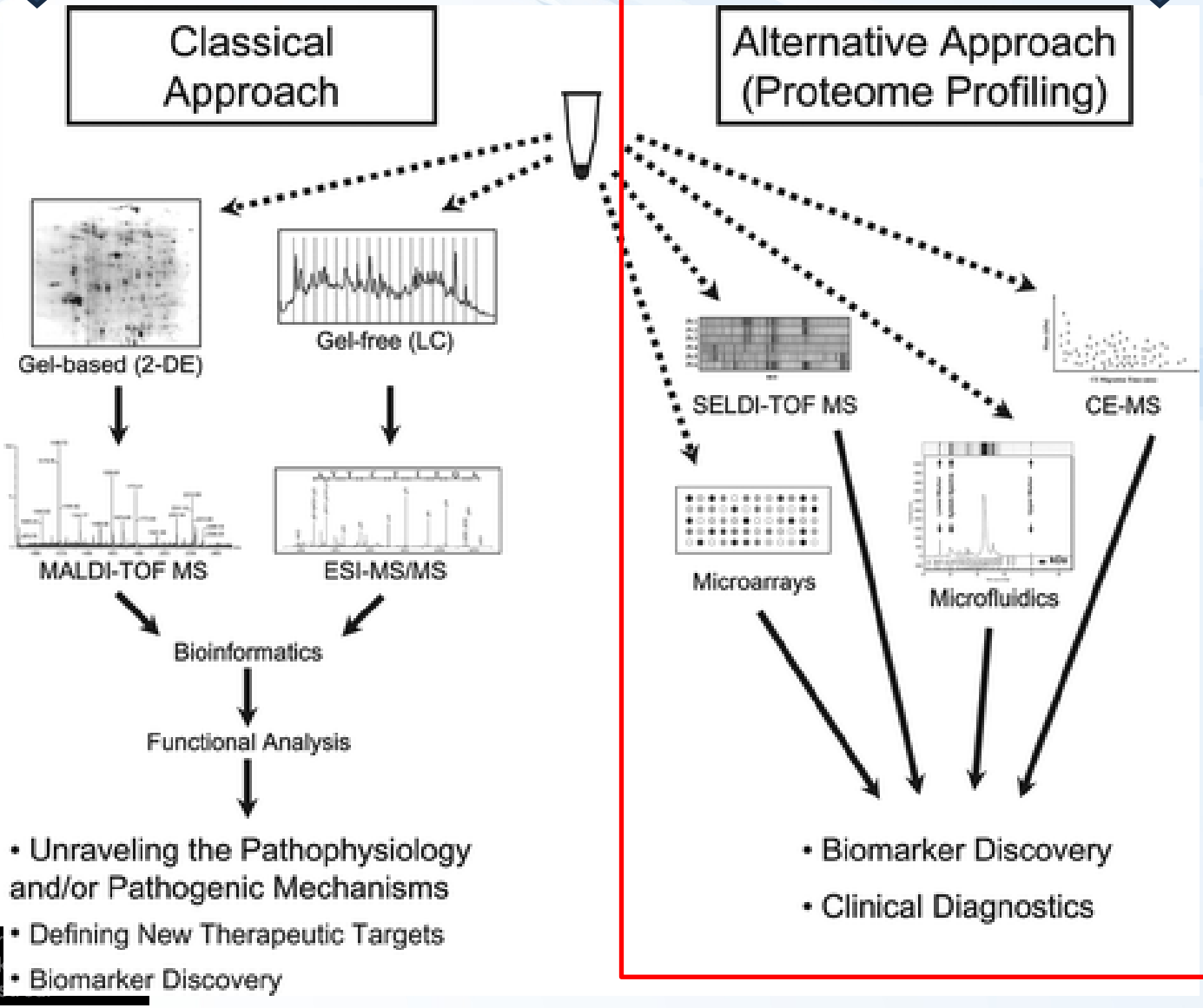
- OTHER "OMICS" (Transcripts, Proteins, Metabolites...)
  - Resposive to environmental stress (including toxicants, therapy etc.)

Biomarkers at different biological levels



Hypothesis driven research (focus on pathways)

Data driven research (omics & profiling)



Biomarkers at **even higher levels** – example: toxic metals

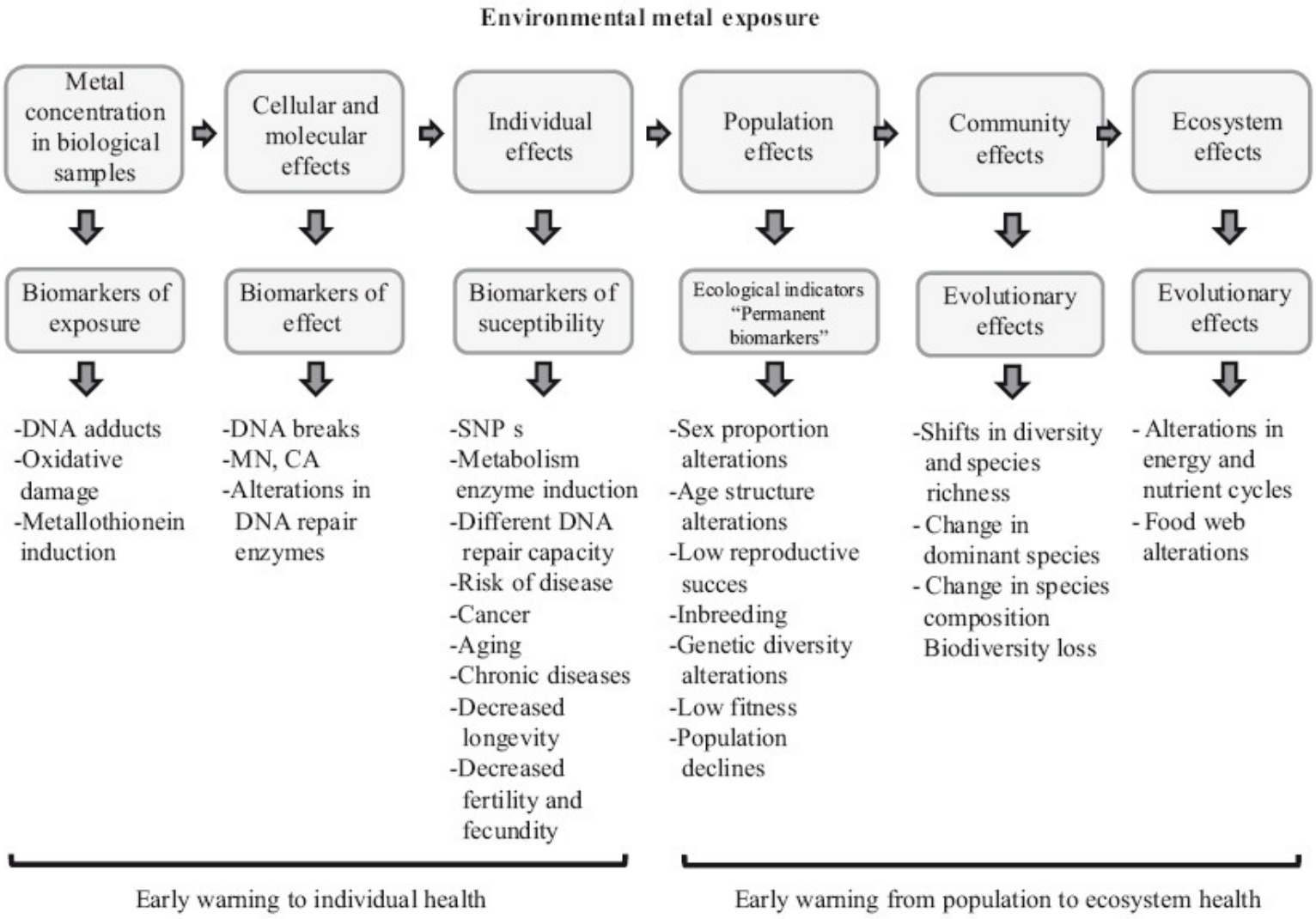


Fig. 1. Environmental pollutants –such as metals– can exert their effects at all levels of biological organization. Most used biomarkers for assessing toxic responses are listed in each level. MN= micronuclei, CA= chromosome aberrations, SNPs= single nucleotide polymorphisms.

Developments and applications of biomarkers

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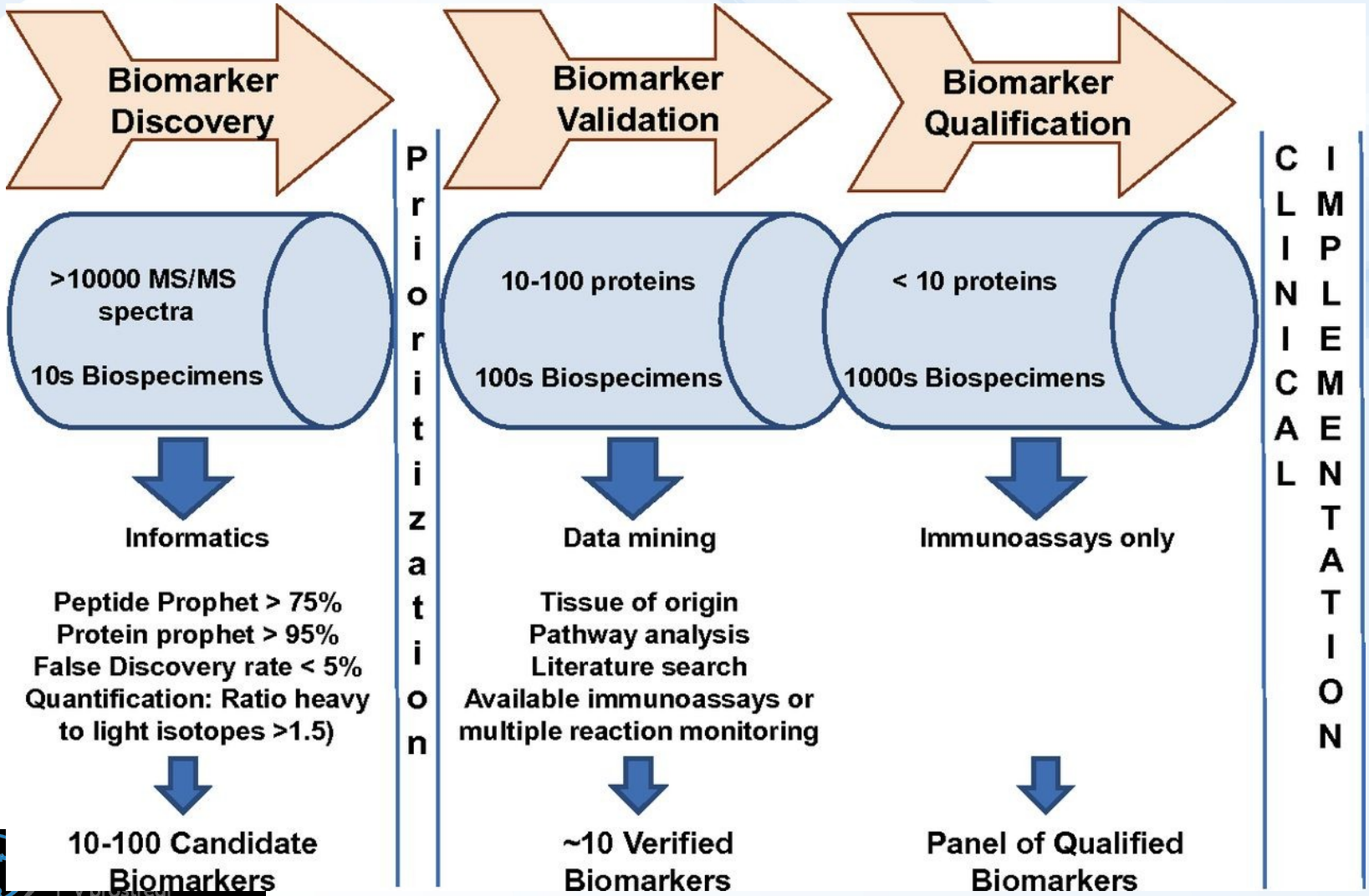
3 key steps towards the biomarker establishment

An example of protein-based biomarkers

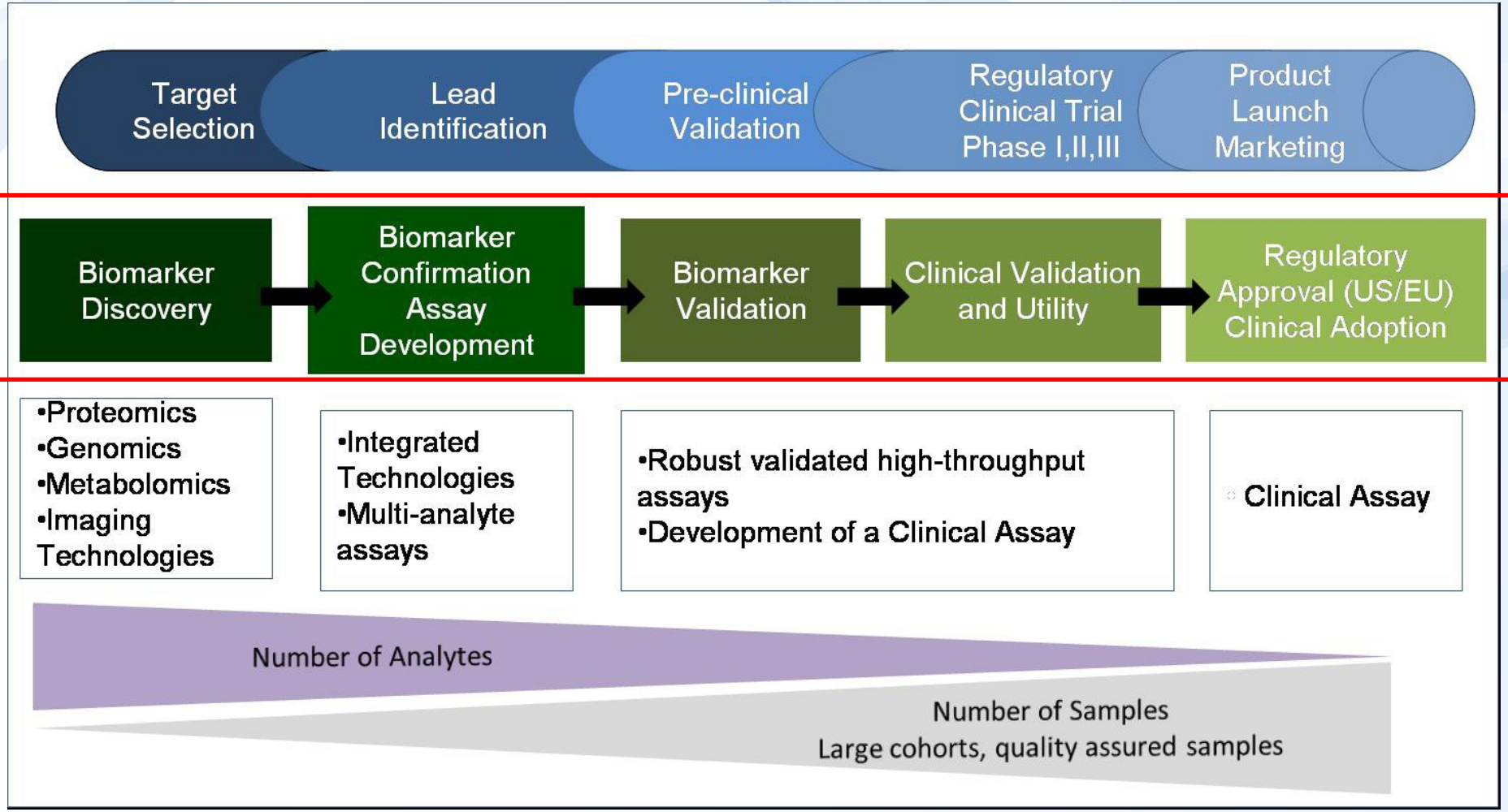


3 key steps towards the biomarker establishment

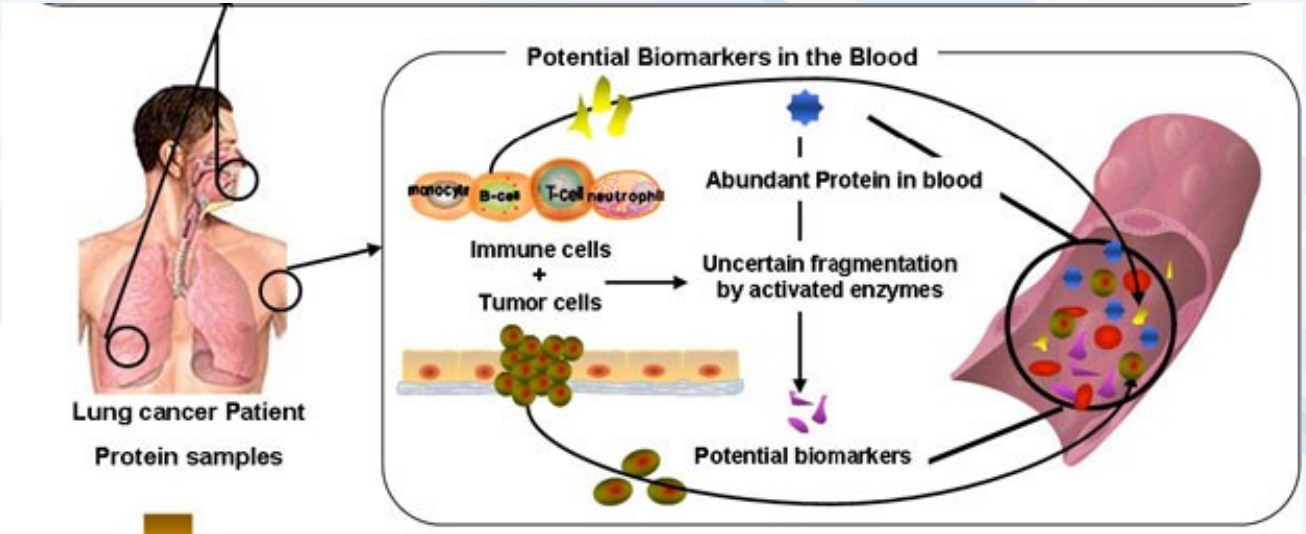
- Biomarker development
  - High numbers of endpoints (e.g. proteins)
  - Low numbers of samples compared (e.g. 10 controls vs 10 “treatments”)
- Biomarker validation
  - Decreasing number of markers
  - Increasing numbers of specimens (biological samples)
- Biomarker qualification and approval
  - Individual markers
  - Analytical methods validated and well established

More detailed view: 5 steps leading to biomarker use in practice

DISCOVERY → VALIDATION STEPS → APPROVAL

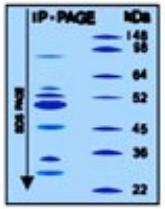


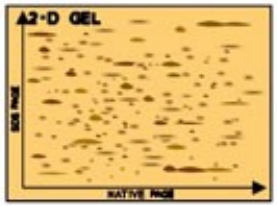
EXAMPLE process of biomarker establishment – lung cancer diagnosis



Protein preparation and separation

- Protein Enrichment: Glycoproteome
  Phosphoproteome
- SDS-PAGE: 1-DE, 2-DE
- In-gel trypsin digestion



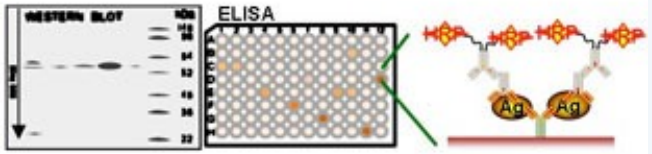


Biomarker discovery

- LC-ESI-MS/MS
- MALDI-TOF/MS

Biomarker candidates verification & validation

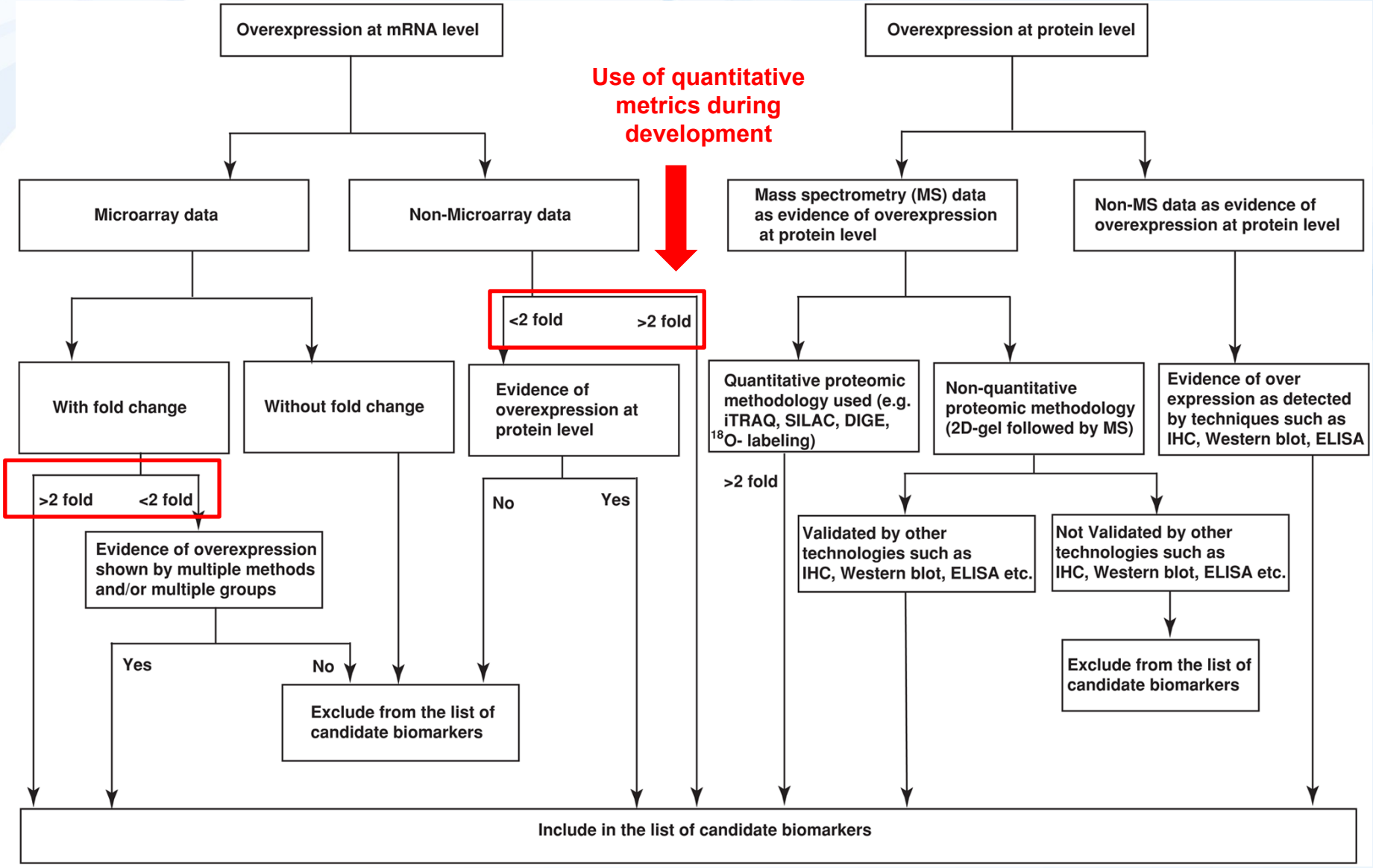
- MRM (Multiple Reaction Monitoring)
- Western Blot
- ELISA



Which of the many changes are “significant” ?

→ Use quantitative metrics
(see Following slide)

What is (what is not) a candidate biomarker: example flowchart



Biomarkers have MANY APPLICATIONS ... such as:

- **Biomarkers in research**
  - Search of “potential” therapies/drugs
    - Changes in biochemical responses provide information on efficiency and mechanism of action
  - Identification of “early markers” of chronic diseases
    - Early diagnosis (e.g. identification of developing cancer, coronary disease...)

- **Biomarkers in medicine**
  - Identification of **status** of an individual
    - Healthy vs Disease
  - Assessment of therapy/treatment
    - Efficiency – Did treatment improved situation? (improvements in biomarker responses)
    - Adverse or side effects of therapy

- **Biomarkers in toxicology**
  - Identification of status
    - Intoxicated (exposed) vs Controls
    - Forensic toxicology (e.g. consumption of drugs of abuse, alcohol etc)
  - Early warnings of future health consequences
    - Biochemical changes are detectable before the actual health problems

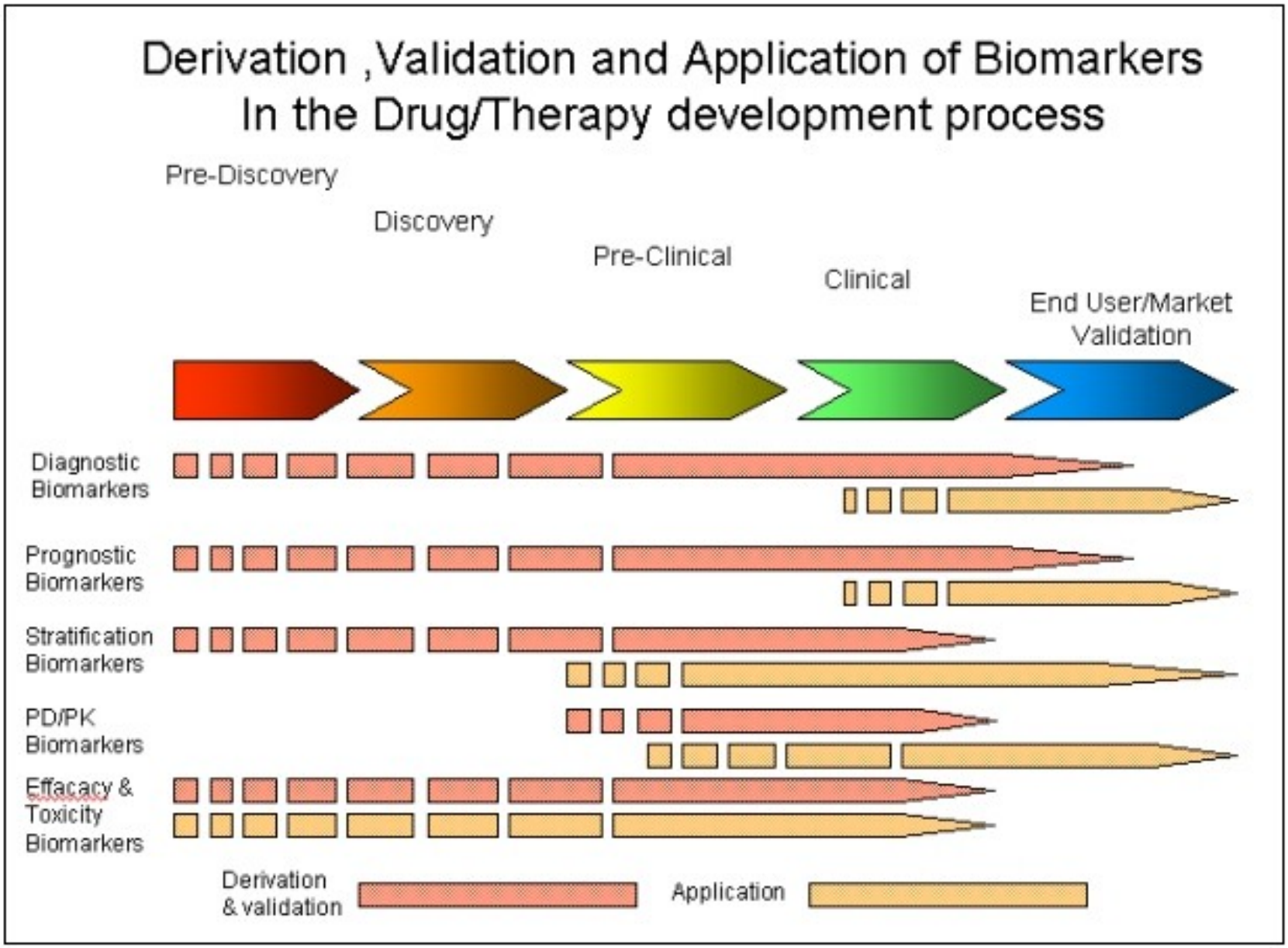
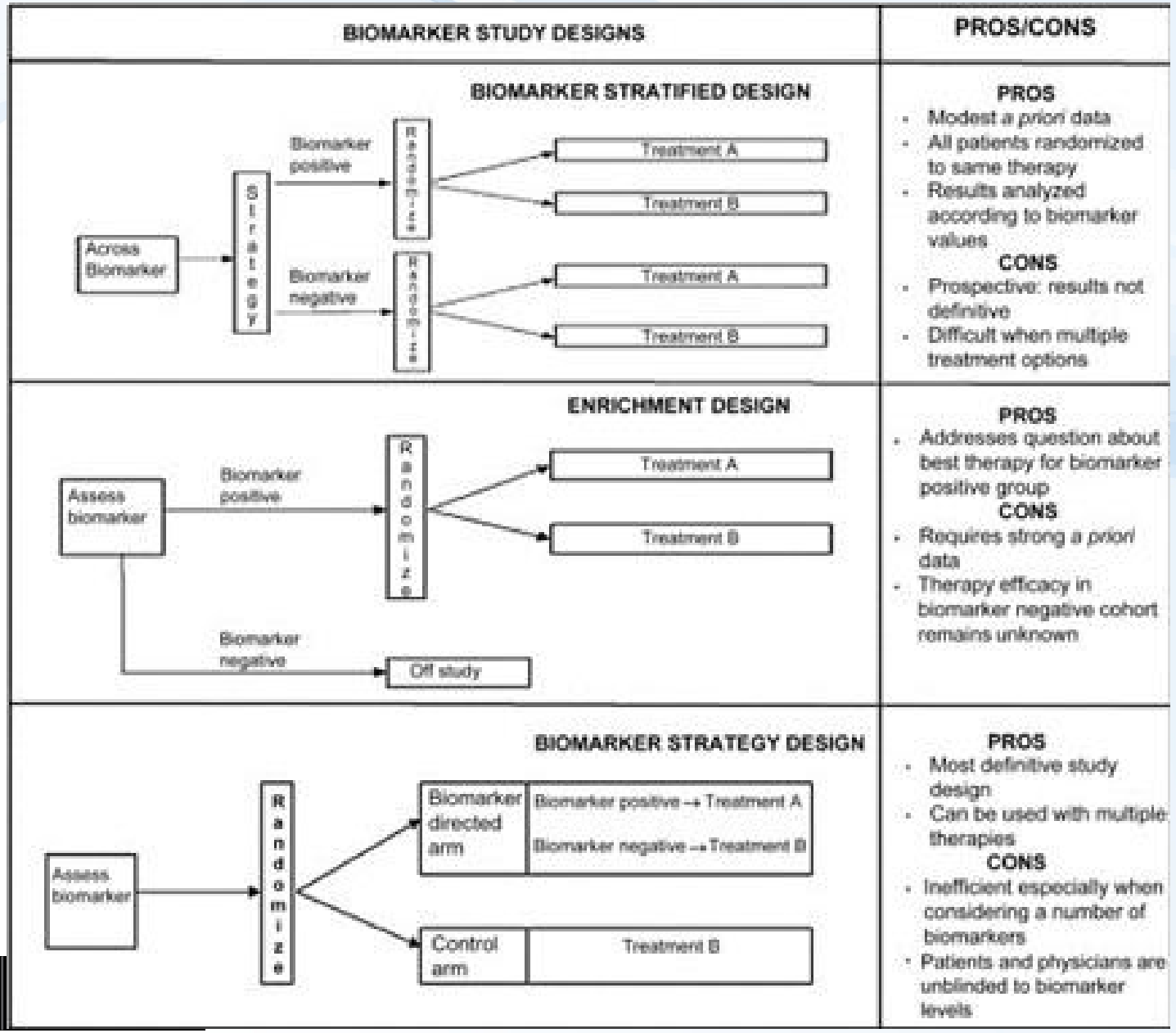


Fig 1 Development and application of biomarkers to the drug development process

Various designs to search for biomarkers of different therapies



Biomarker applications in CLASSIFICATION OF CARCINOGENS

TABLE 20.1 Summary of IARC changes in carcinogenicity status based on biomarker data.

Change	Number of Chemicals
Mechanistic evidence used to upgrade hazards from 2A (probably carcinogenic to humans) to 1 (carcinogenic to humans)	3
Mechanistic evidence used to upgrade hazards from 2B (possibly carcinogenic to humans) to 2A (probably carcinogenic to humans)	36
Mechanistic evidence used to upgrade hazards from 3 (not classifiable as to carcinogenicity to humans) to 2B (possibly carcinogenic to humans)	4
Mechanistic evidence used to downgrade hazards from 2B (possibly carcinogenic to humans) to 3 (not classifiable as to carcinogenicity to humans)	8
Total	51

IARC – International Agency for Research of Cancer

Biomarker validation EXAMPLE

Kim-1 protein levels and kidney clinical signs (histopathology grades 0-3)

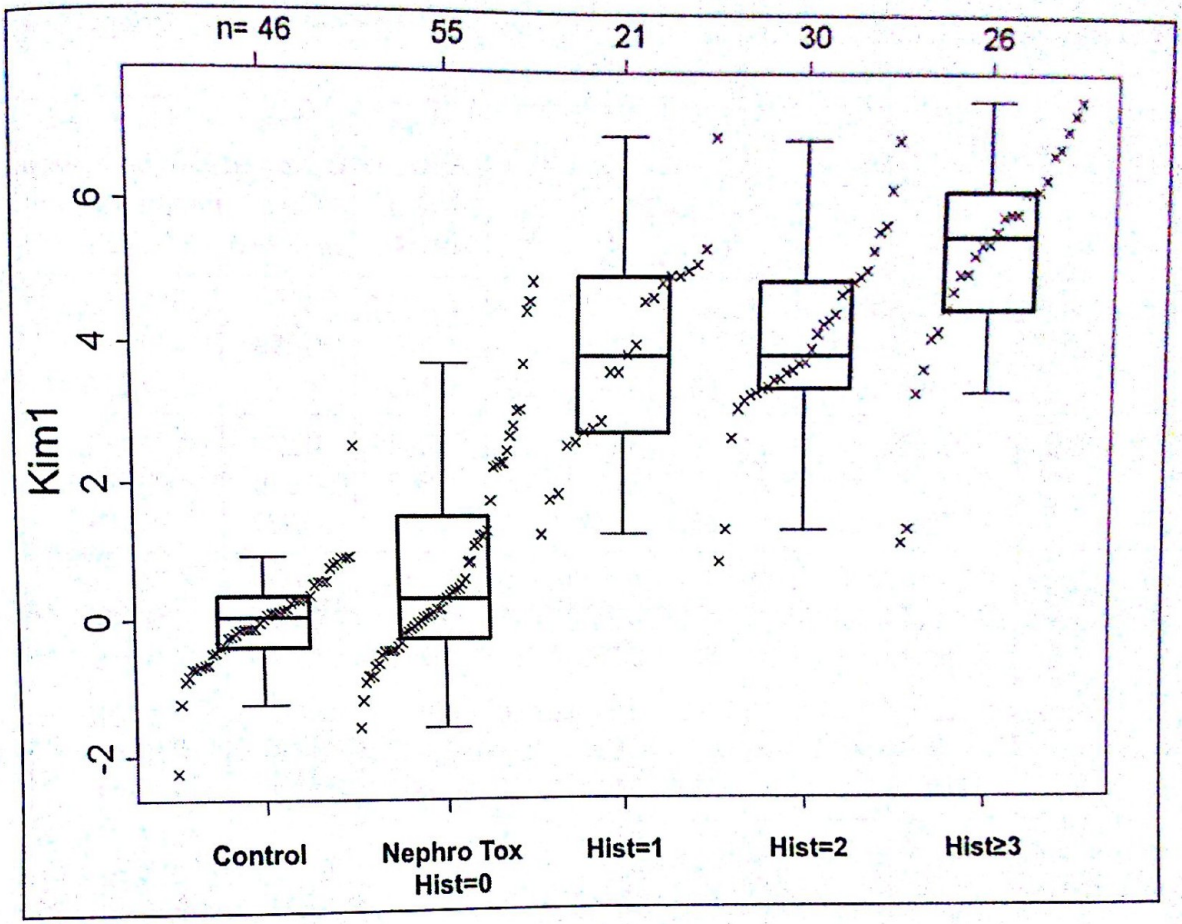
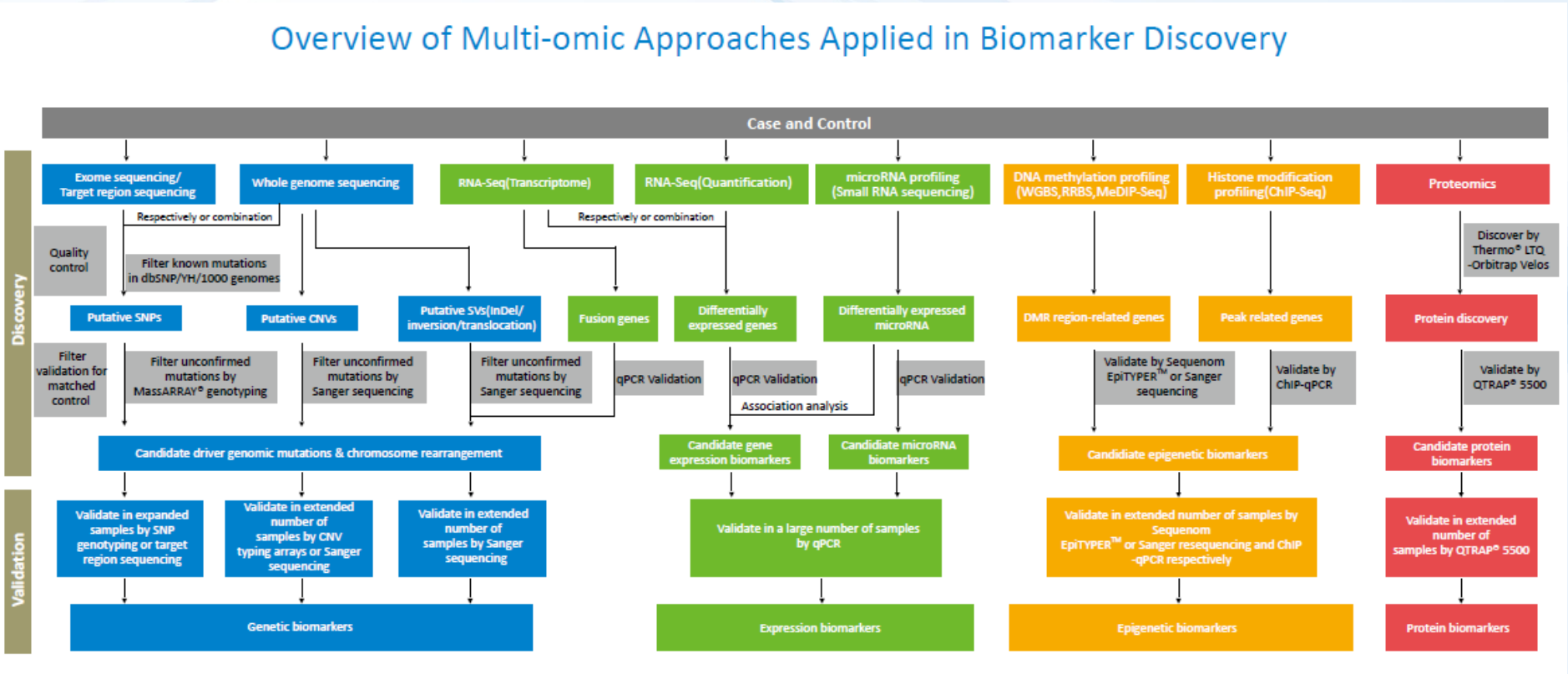


FIGURE 22.4 Boxplots of Kim-1 values by kidney histopathology injury grade. A plot of the individual values sorted by Kim-1 value is superimposed over each, giving a finer scaled picture of the distribution of the data. The figure indicates that median Kim-1 values generally increase with an increased histopathology score. Also, some samples in the group of animals treated with a nephrotoxicant but with histopathology scores of zero have elevated Kim-1 levels. (See color insert for a full color version of this figure.)

OMICS biomarkers in discovery and validation



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toxicity mechanisms (MoA) and biomarkers

overall summary

Molecular effects of toxicants (1)

→ Propagate to higher levels (2),

→ here they induce various “responses” detectable as (3) biomarkers

1

MoAs

<u>* Molecular interactions</u>

* Key targets ...:
  - DNA, RNAs
  - proteins (and their functions)
  - membranes

<u>* Complex mechanisms</u>
  - Oxidative stress
  - Signalling and hormones
  - Detoxification

2

Biological organization

3

Biomarkers

- types
- examples
- methods

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