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 have MANY APPLICATIONS ... such as:

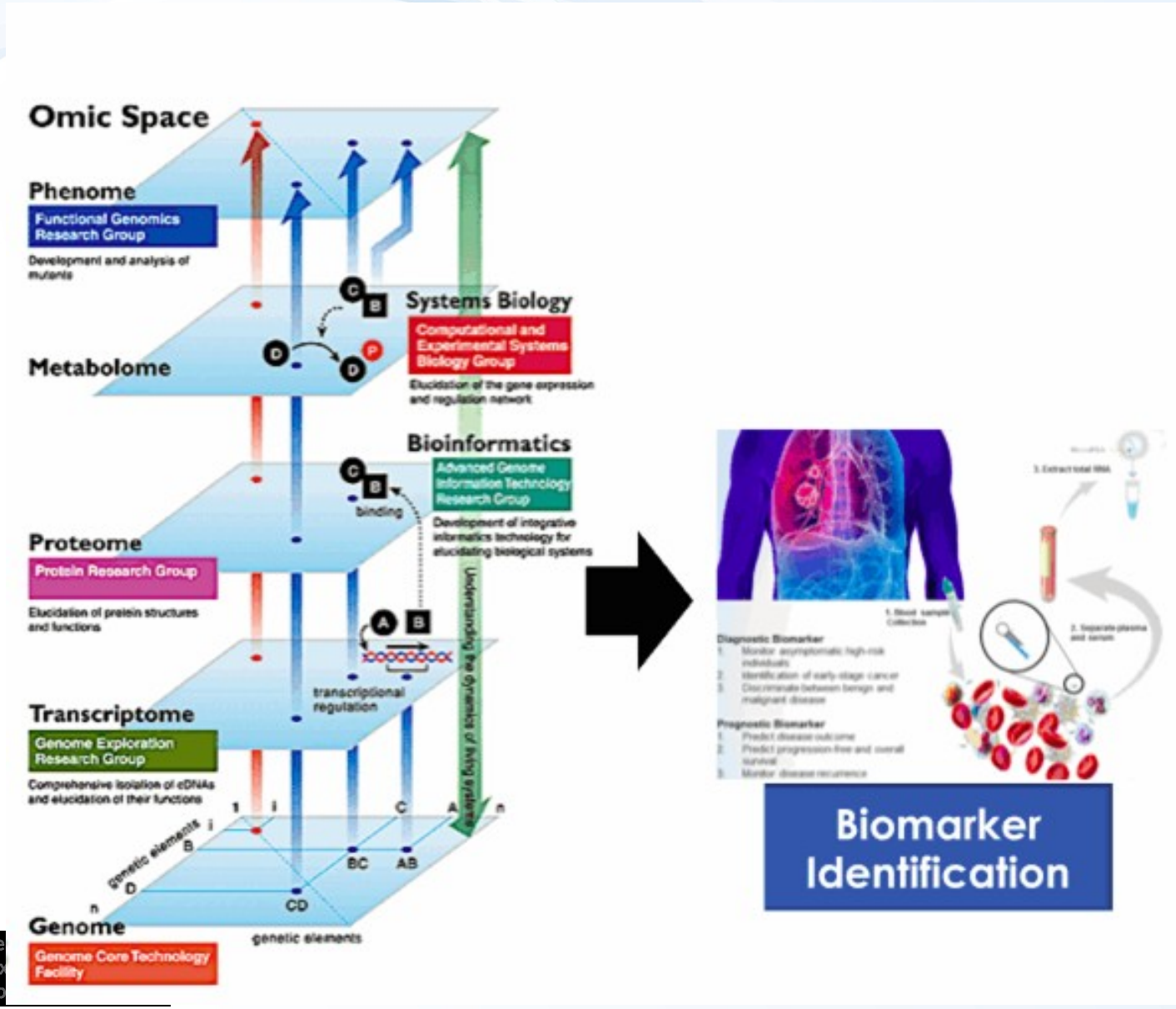
- **Biomarkers in research**
  - Search of “potential” therapies/drugs
    - Changes in biochemical responses provide information on efficiency and mechanism of action
  - Identification of “early markers” of chronic diseases
    - Early diagnosis (e.g. identification of developing cancer, coronary disease...)

- **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

Biomarkers at various levels “omics”

Biomarkers at different biological levels – „omics“ approach



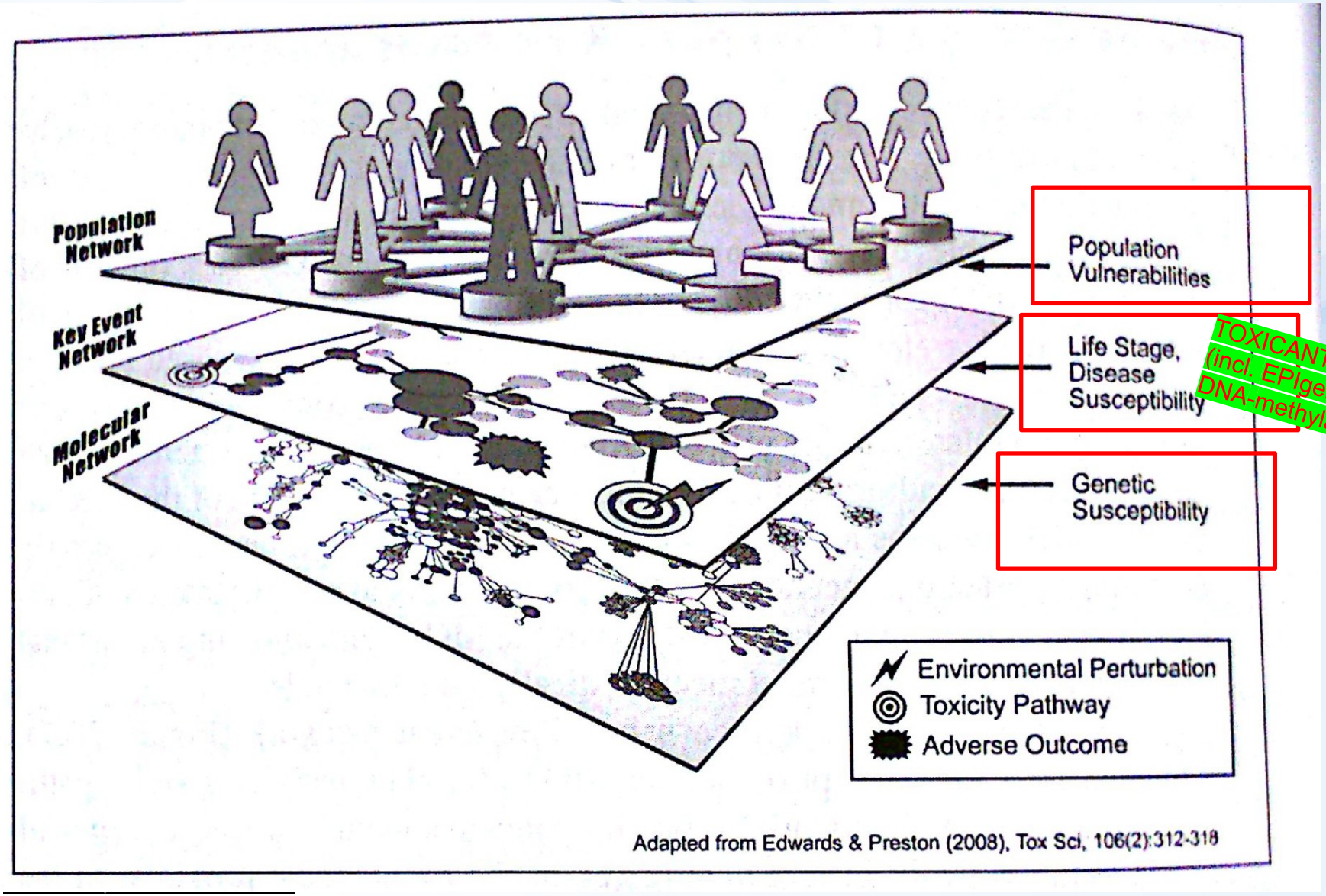
Biomarkers at different biological levels

- **"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)

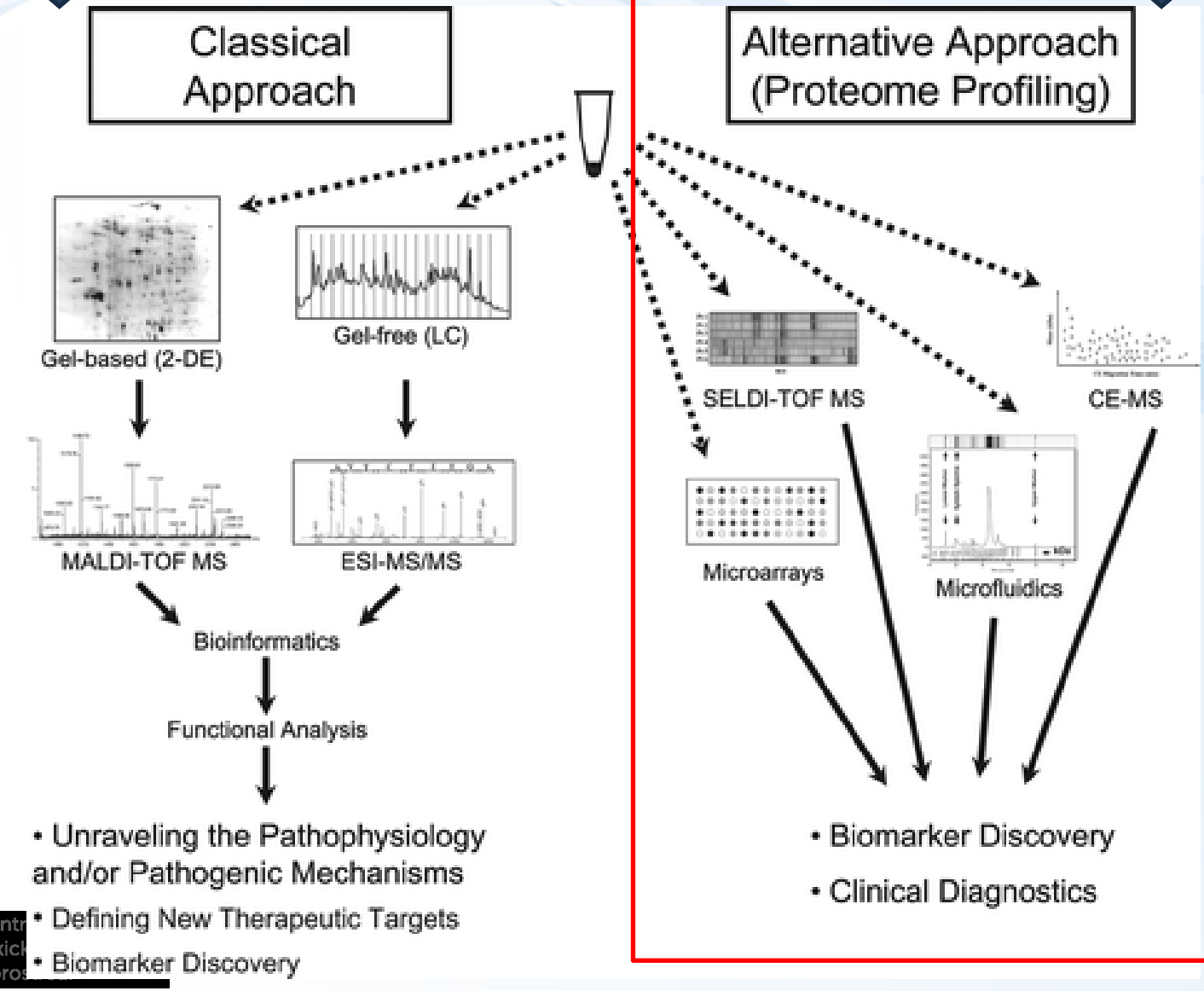
- **OTHER "OMICS" layers**
  - Epigenomics + Transcriptomics, Proteo-, Metabolo-...
  - Highly variable - responsive to environmental stress (including toxicants, therapy etc.)

Biomarkers at different biological levels



Hypothesis driven research (focus on pathways)

Data driven research (omics & profiling)



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

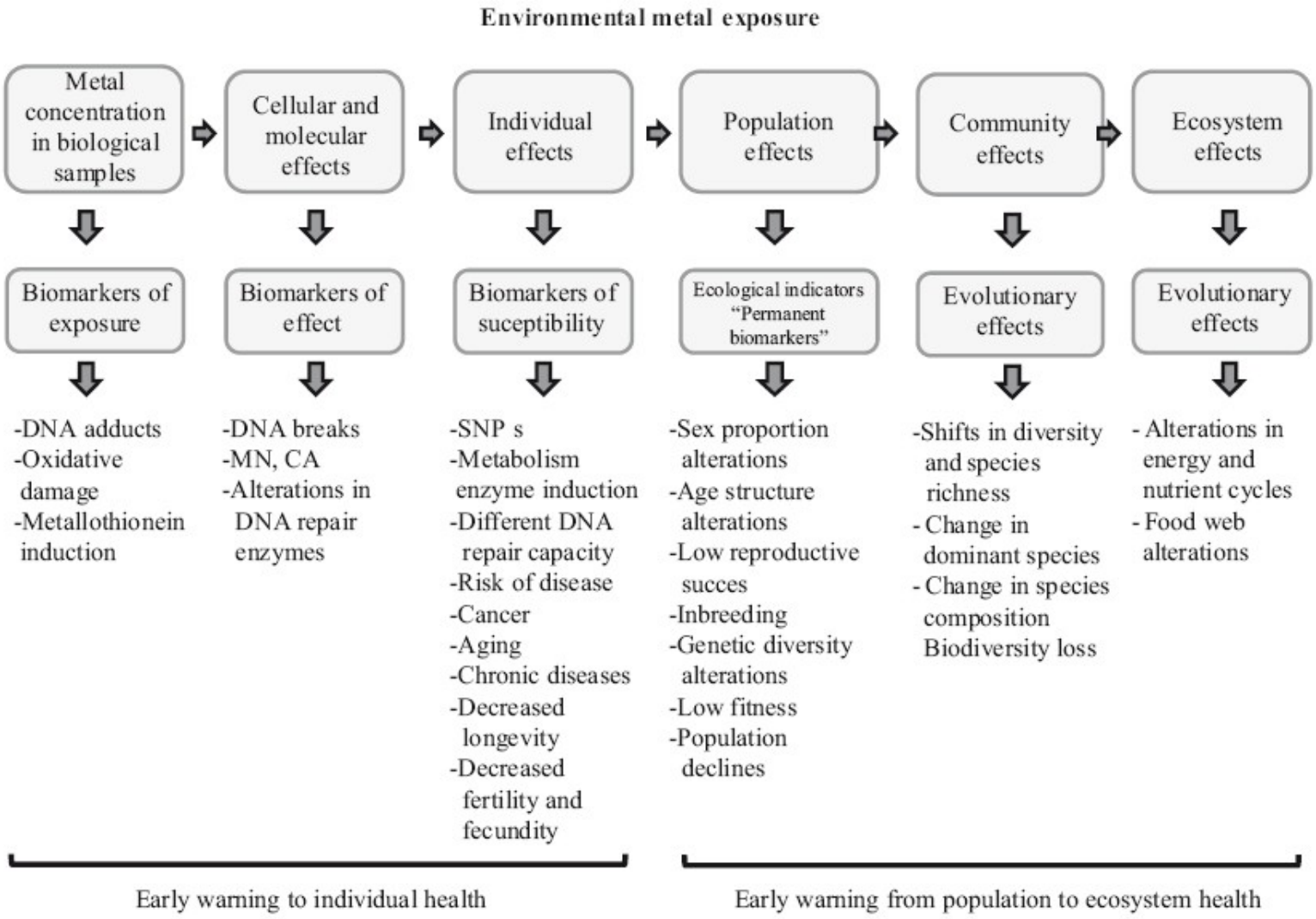


Fig. 1. Environmental pollutants –such as metals– can exert their effects at all levels of biological organization. Most used biomarkers for assessing toxic responses are listed in each level. MN= micronuclei, CA= chromosome aberrations, SNPs= single nucleotide polymorphisms.

Developments and applications of **clinical** biomarkers

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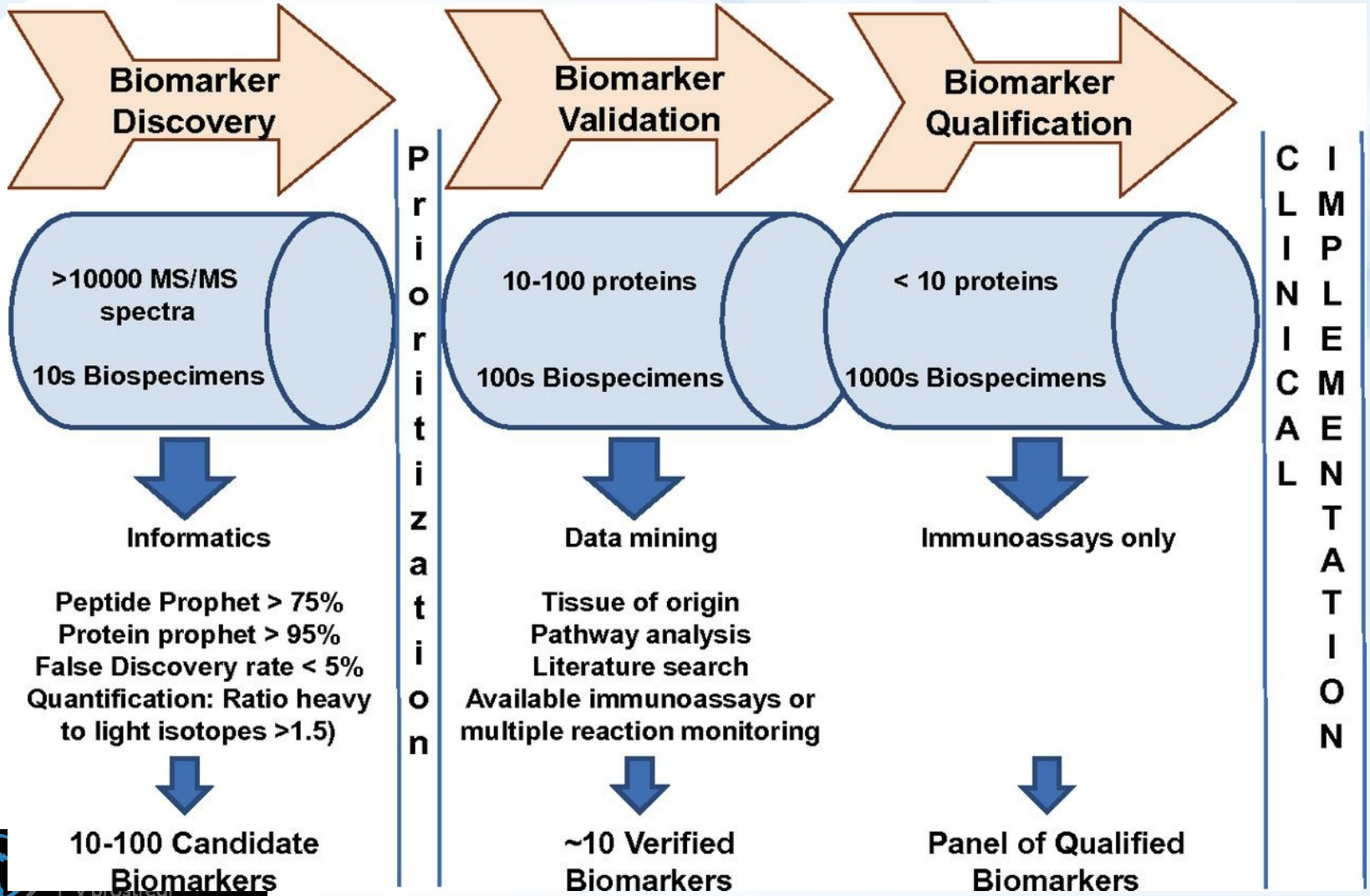
3 key steps towards the biomarker establishment

An example of protein-based biomarkers

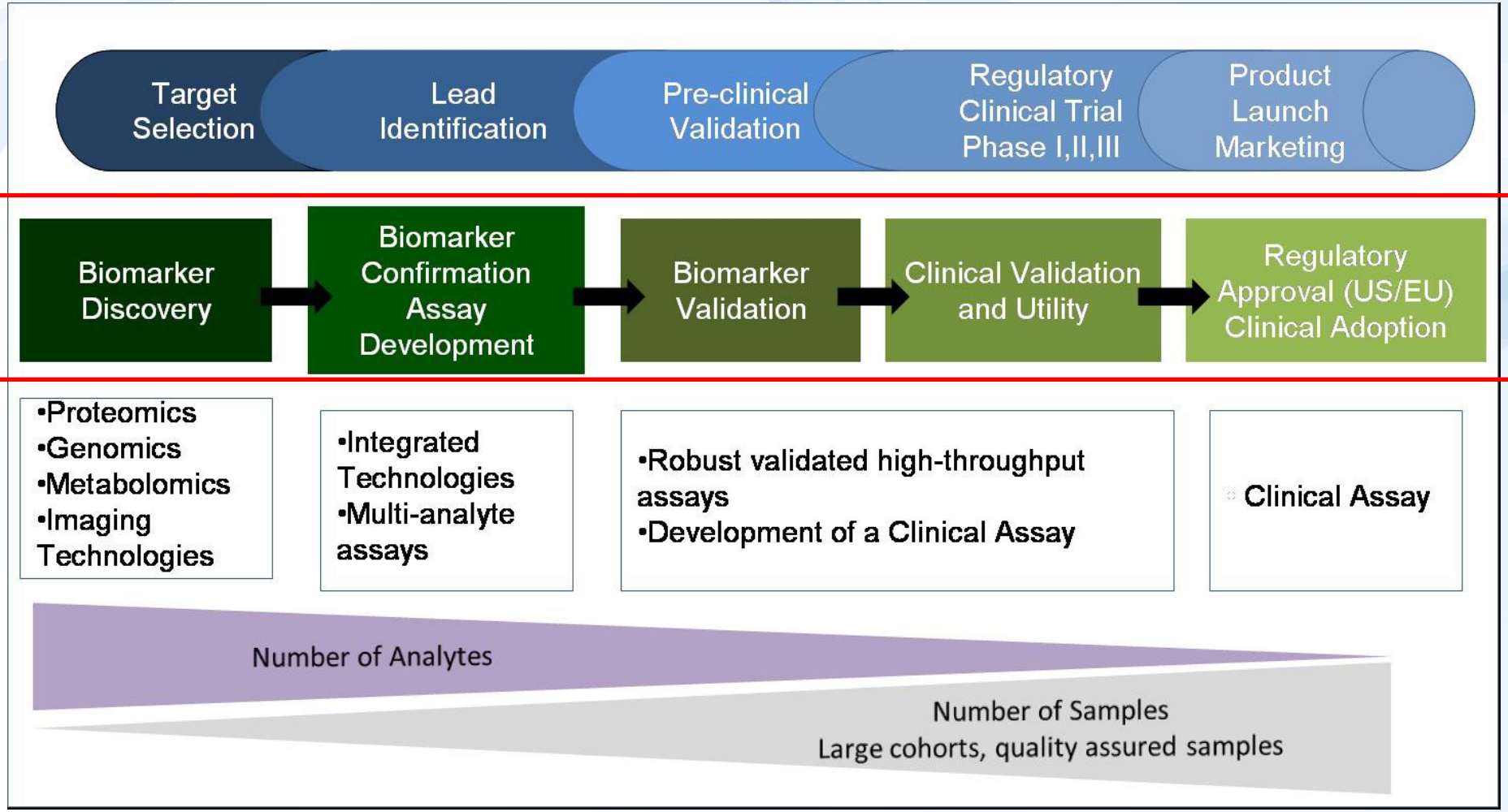


3 key steps towards the biomarker establishment

- **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 (clinical) practice

DISCOVERY → VALIDATION STEPS → APPROVAL



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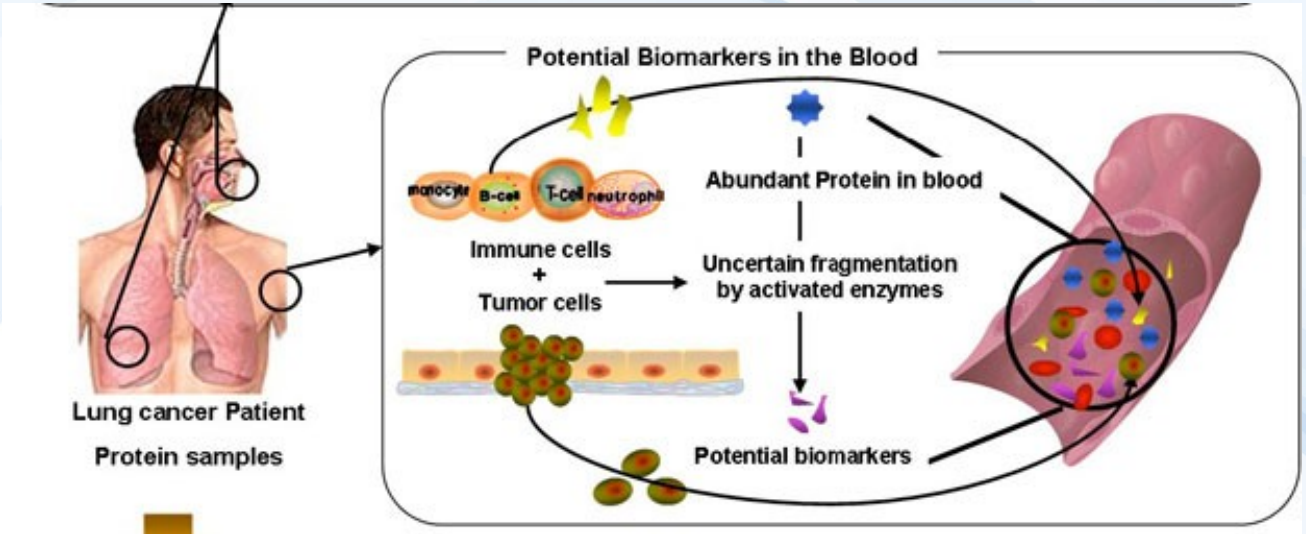
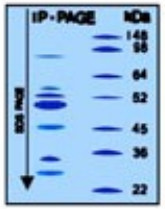
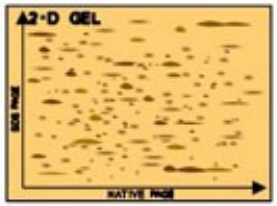
EXAMPLE process of biomarker establishment – lung cancer diagnosis



Protein preparation and separation

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



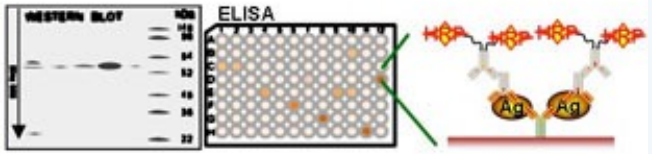


Biomarker discovery

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

Biomarker candidates verification & validation

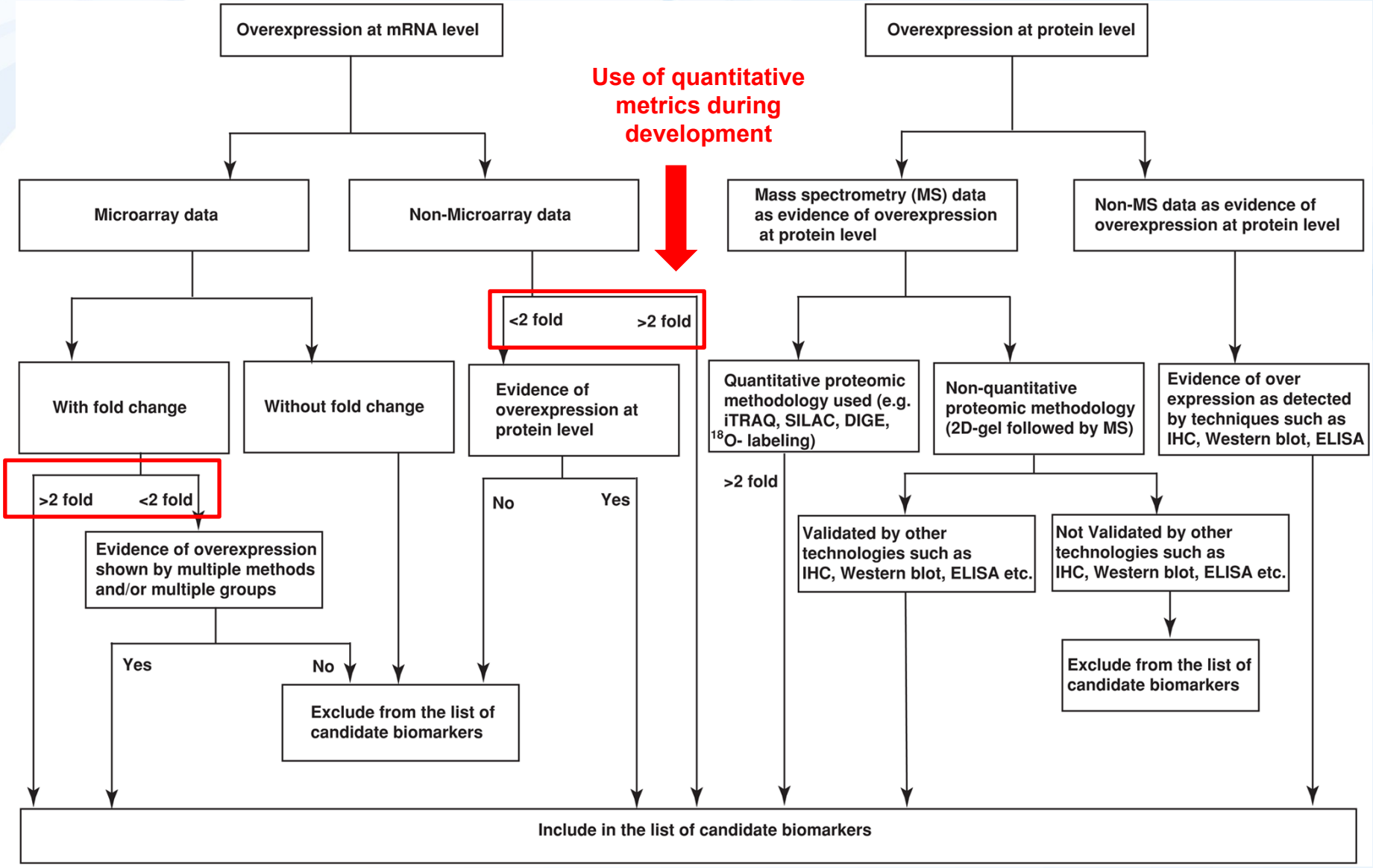
- MRM (Multiple Reaction Monitoring)
- Western Blot
- ELISA



Which of the many changes are “significant” ?

→ Use quantitative metrics
(see Following slide)

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



Biomarker validation EXAMPLE

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

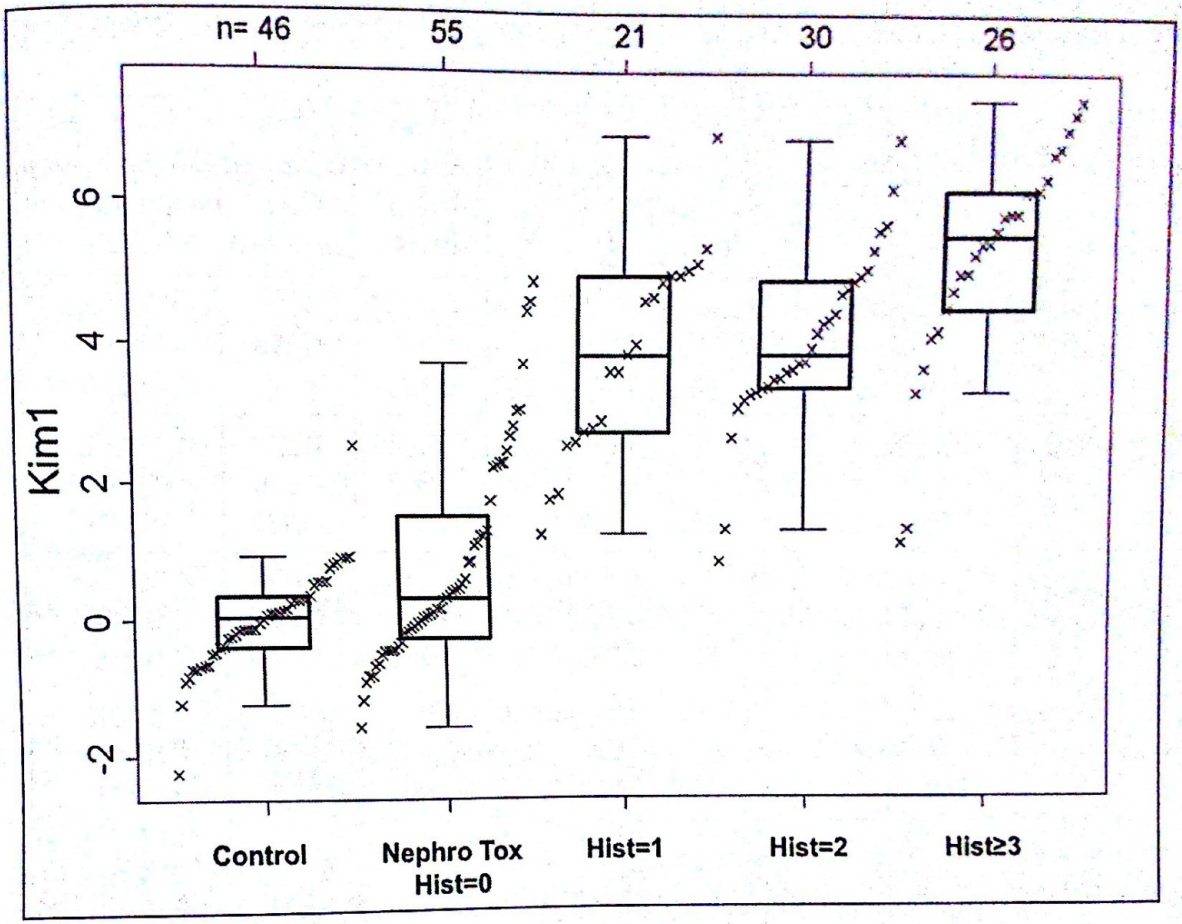
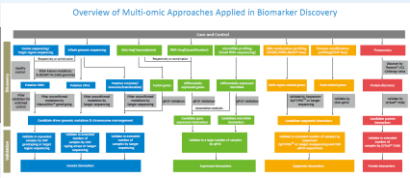
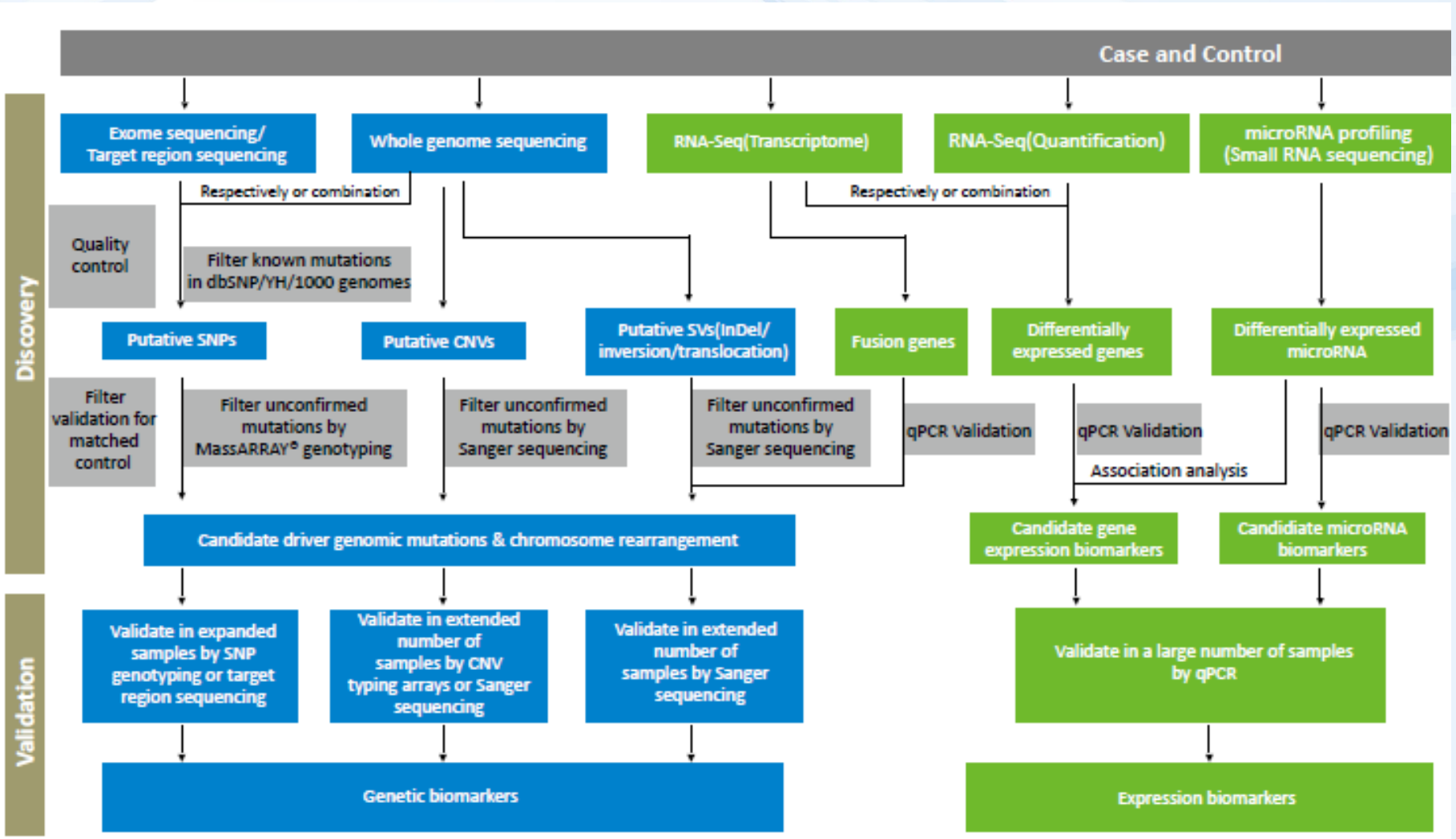


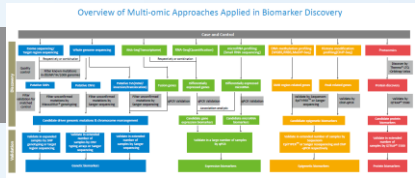
FIGURE 22.4 Boxplots of Kim-1 values by kidney histopathology injury grade. A plot of the individual values sorted by Kim-1 value is superimposed over each, giving a finer scaled picture of the distribution of the data. The figure indicates that median Kim-1 values generally increase with an increased 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.)

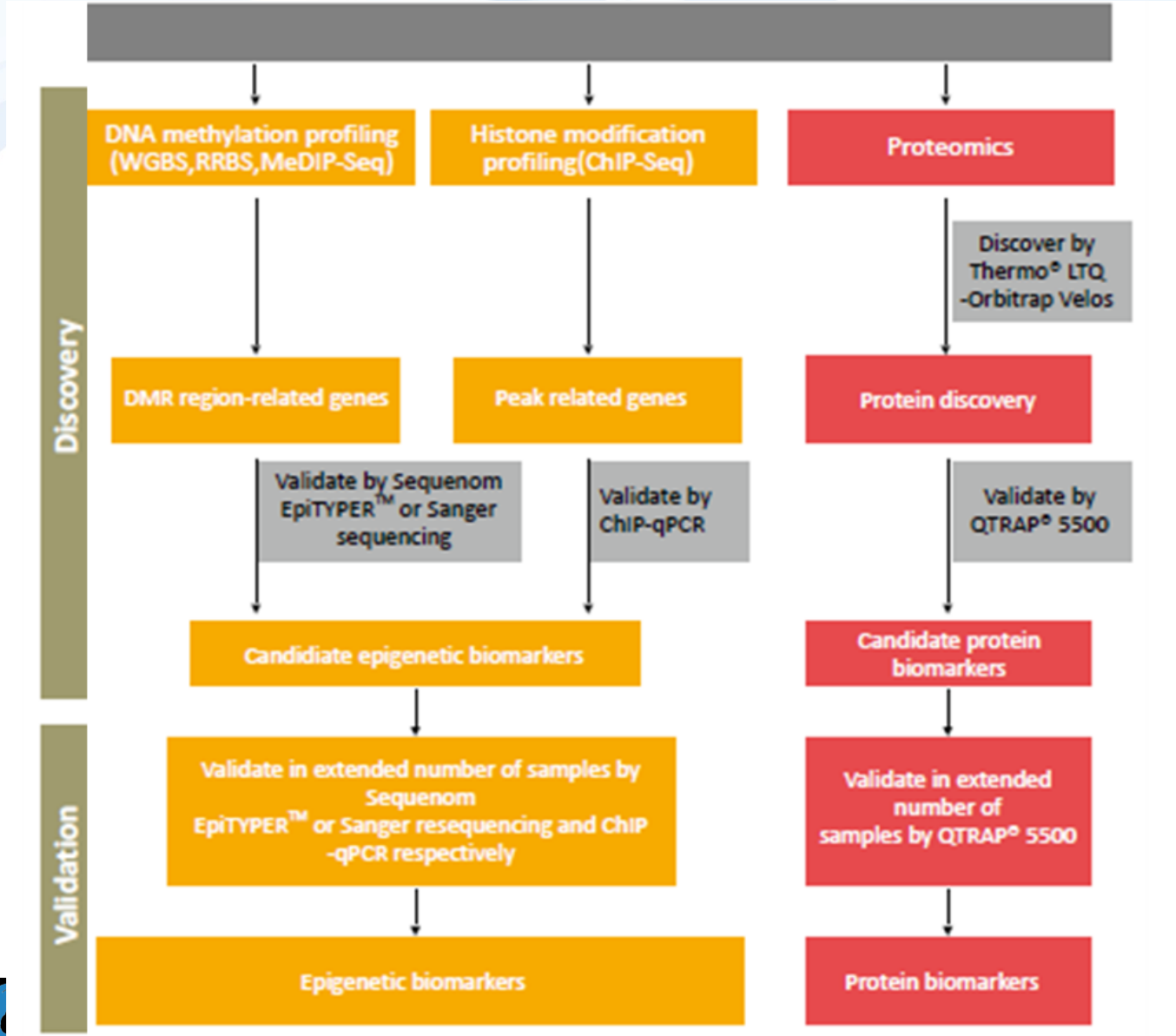
OMICS biomarkers in discovery and validation (1/2)





OMICS biomarkers in discovery and validation (2/2)





Summary and overview

Toxicity mechanisms (MoA) and biomarkers

Class summary and take home message

* Molecular effects of toxicants = MoAs (1)
* Propagate to higher levels (2),
* … where they induce measurable “responses” - biomarkers (3)

1

MoAs

* Molecular interactions
* Key targets ...:
  - DNA, RNAs
  - proteins (and their functions)
  - membranes
* Complex mechanisms
  - Oxidative stress
  - Signalling and hormones
  - Detoxification

2

Biological organization

3

Biomarkers

- types
- examples
- methods

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Summary on toxicity mechanisms (MoA) and biomarkers

For excellent performance and successful exam student should:

1. have an **overview** of different types of MoAs (see also point 2 below) and be able to **link** MoAs to higher level effects (toxicity)
   - *Example: What is the in vivo manifestation (effect) after inhibition of AcCholE enzymes (mechanism)? [AcCholE inhibition propagates as neurotoxicity (effect)]*

   Be ready to discuss also in a opposite way
   - *Example: What MoA can be beyond immunotoxicity? [Immunotoxicity can e.g. be caused by disruption of signaling pathways – LPS as an example]*

2. know some **details for selected example MoAs** for different toxicant targets
   = based on your own preference select one example from the following 7 categories, learn details, and be ready to discuss (i.e. learn details for 1 out of 7 example modes of toxic action)
   1. nucleic acids
   2. proteins
   3. membranes (lipids)
   4. cellular
   5. Complex 1 – detoxification/metabolization
   6. Complex 2 – intra- and inter-cellular signalling, hormones
   7. Complex 3 – oxidative stress

3. have **understanding of biomarker** issues
   - What is a biomarker and what properties it should have (or not to have)?
   - Why we search for them = how can they be used?
   - What different types and groups of biomarkers can be recognized?
   - What are suitable matrices for sampling and further analyses?
   - What methods do we use for analyses of biomarkers? (LCMS, ELISA, PCR, Proteins-WBs, Enzyme actvities)
   - What approaches are applied in biomarker discovery („hypothesis“ vs omics)?

4. and **know example biomarkers**
   Related to the point 2 above
   = based on your own interest (in point 2) learn about the effect biomarkers relevant for your selected toxicity mechanism

The final exam:
= 4 open questions + follow-up „colloquium-like“ discussion

Q1 + Q3 – general overview

Q2 + Q4 – select one topic from those listed (1-7) and know details about mechanisms & biomarkers