



AGAH
Arbeitsgemeinschaft
für angewandte
Humanpharmakologie e.V.

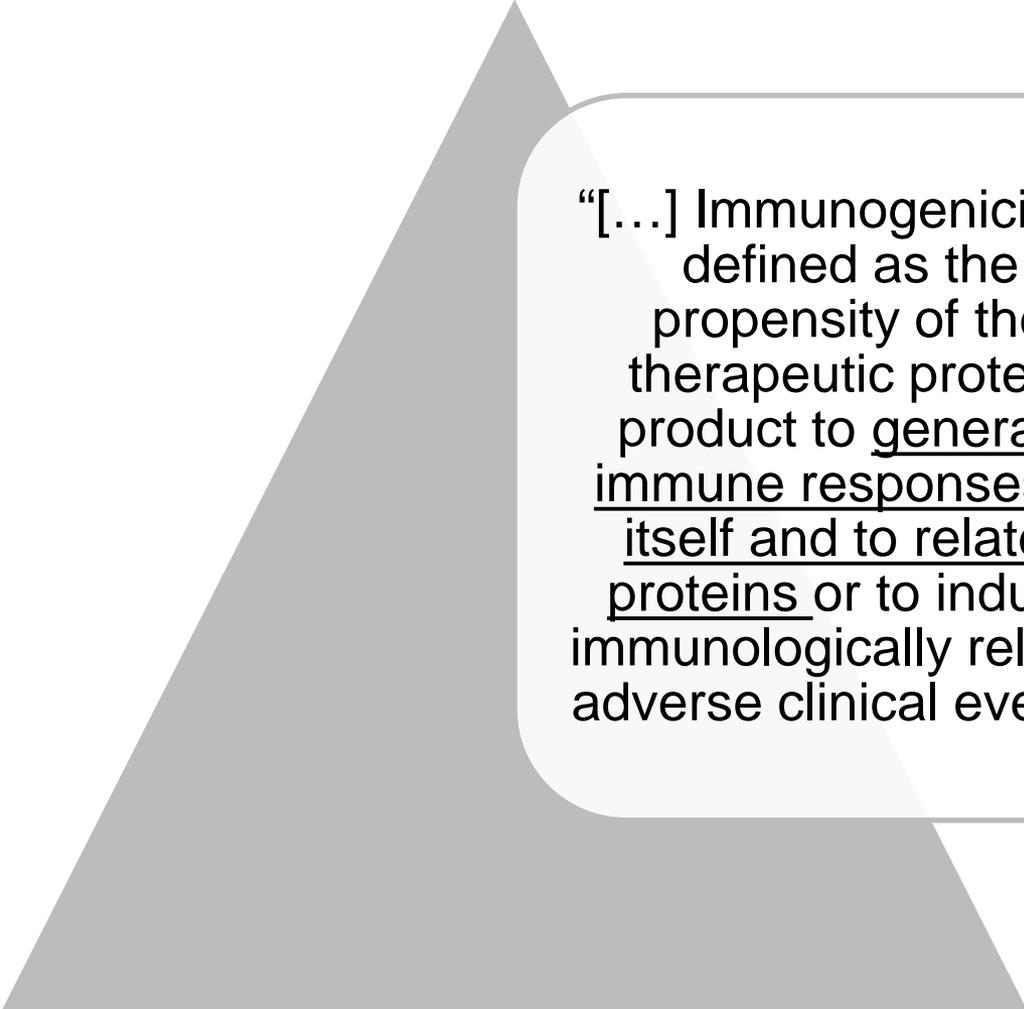
Immunological Aspects in the Non-Clinical Safety Assessment of Drugs in Development

Stephanie Plassmann

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Definitions and Key Aspects

Immunogenicity (FDA 2014)

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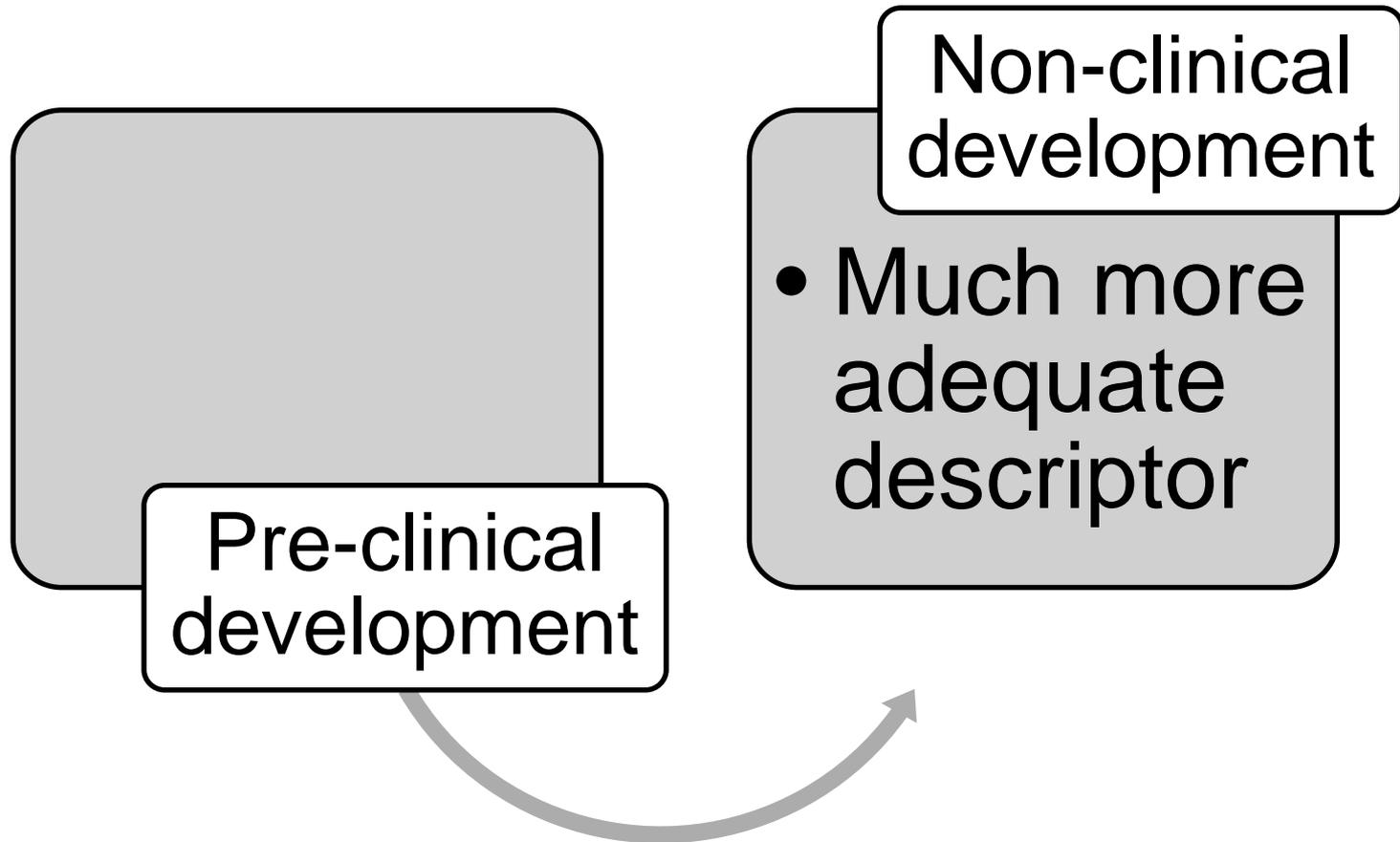
“[...] Immunogenicity is defined as the propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events”

“From a regulatory point of view, the predictive value of non-clinical studies for evaluation of immunogenicity of a biological medicinal product in humans is low due to differences between human and animal immune systems and to immunogenicity of human proteins in animals. While non-clinical studies aimed at predicting immunogenicity in humans are normally not required, novel models may, for example, be of value in selecting lead compounds for development and unravelling cellular mechanisms.”

“Immunotoxicity is, for the purpose of this guideline, defined as unintended immunosuppression or enhancement. Drug-induced hypersensitivity and autoimmunity are excluded.”

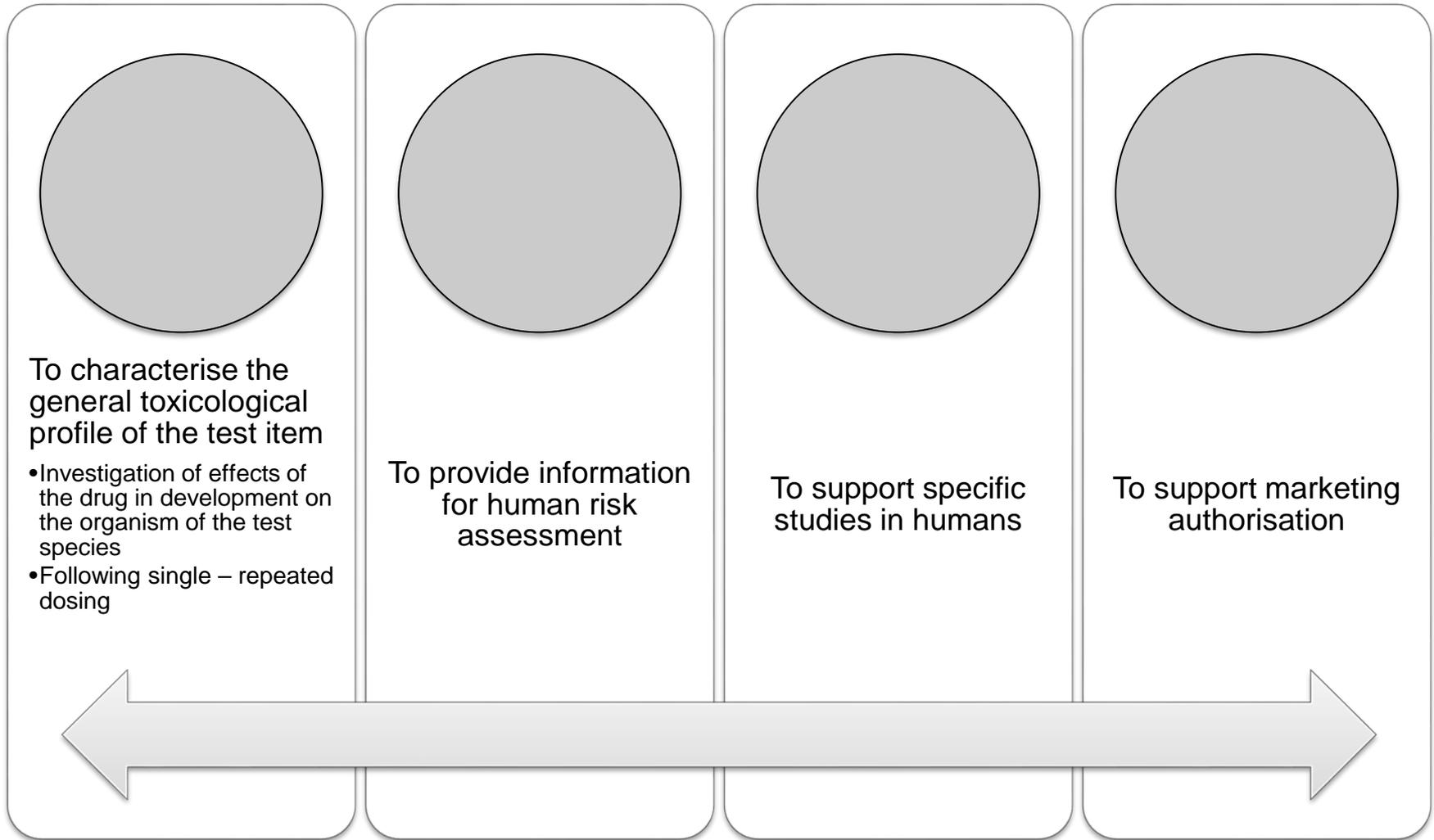
“One aspect of immunotoxicological evaluation includes assessment of potential immunogenicity”

Preclinical and clinical development are closely intertwined from start to end

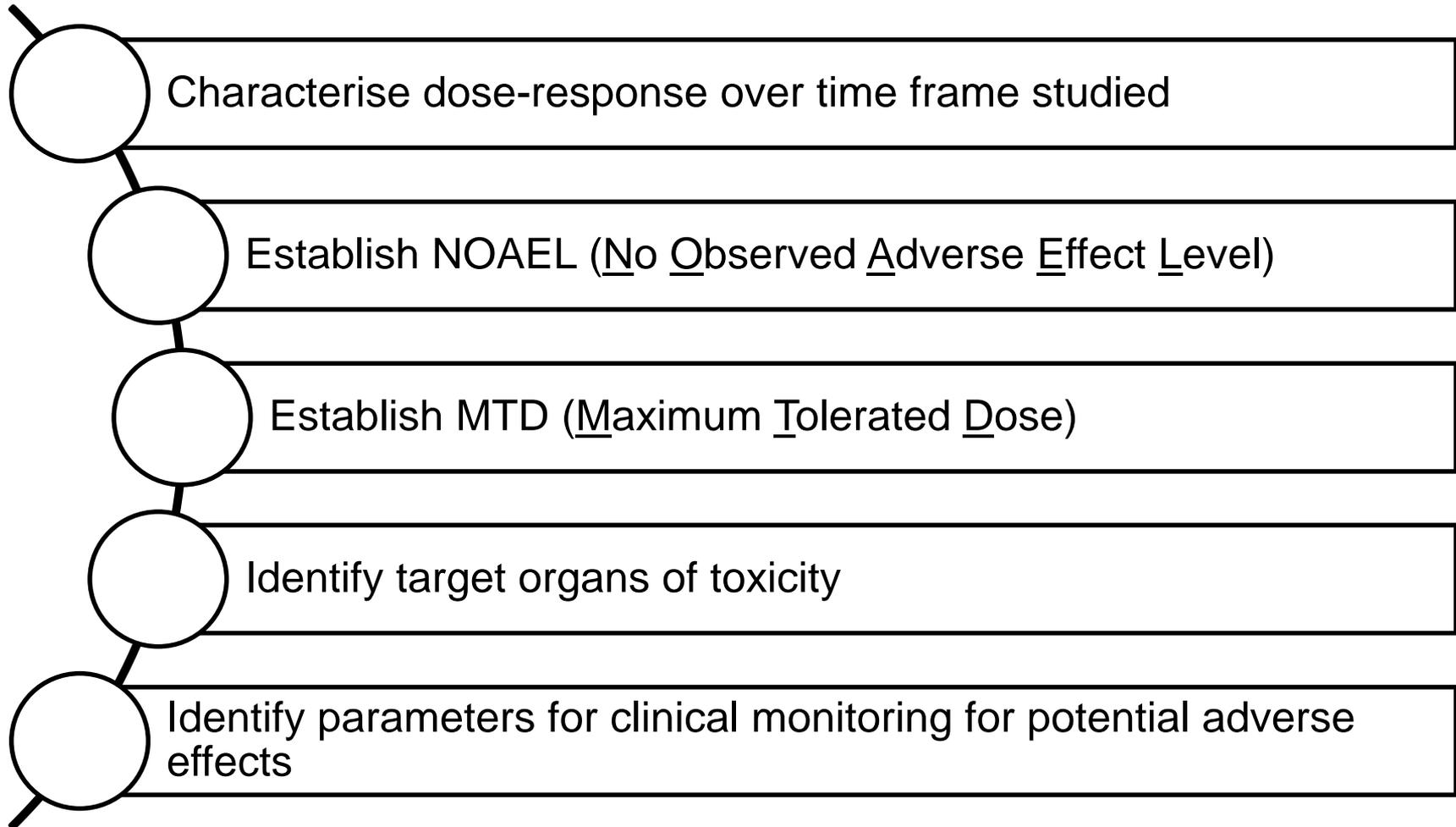


General Toxicology

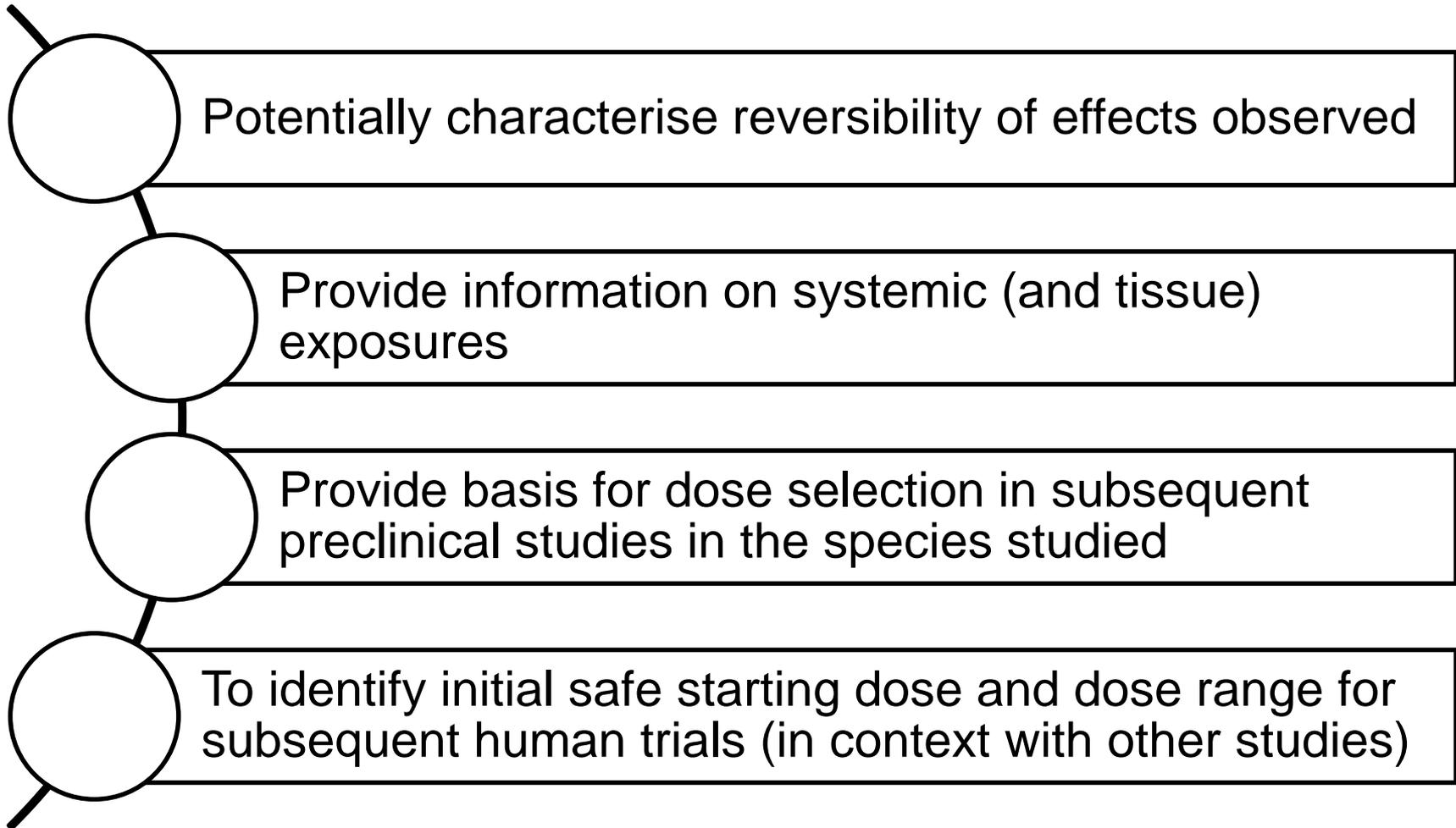
Primary objectives



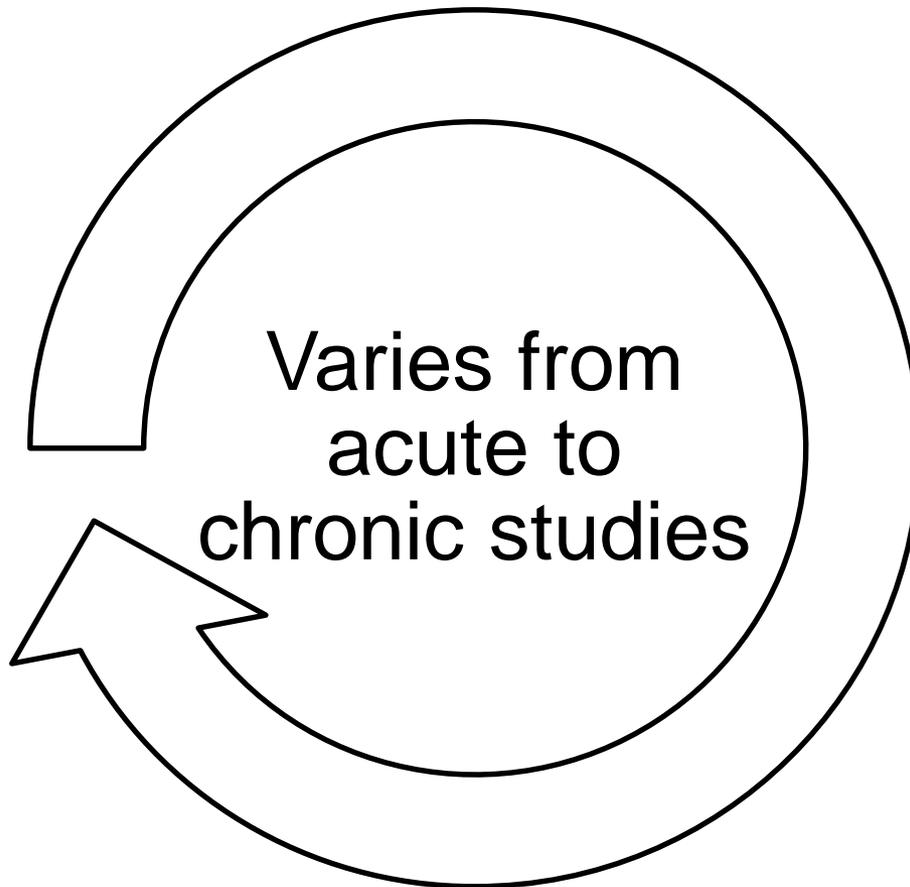
Principal Aims (1)



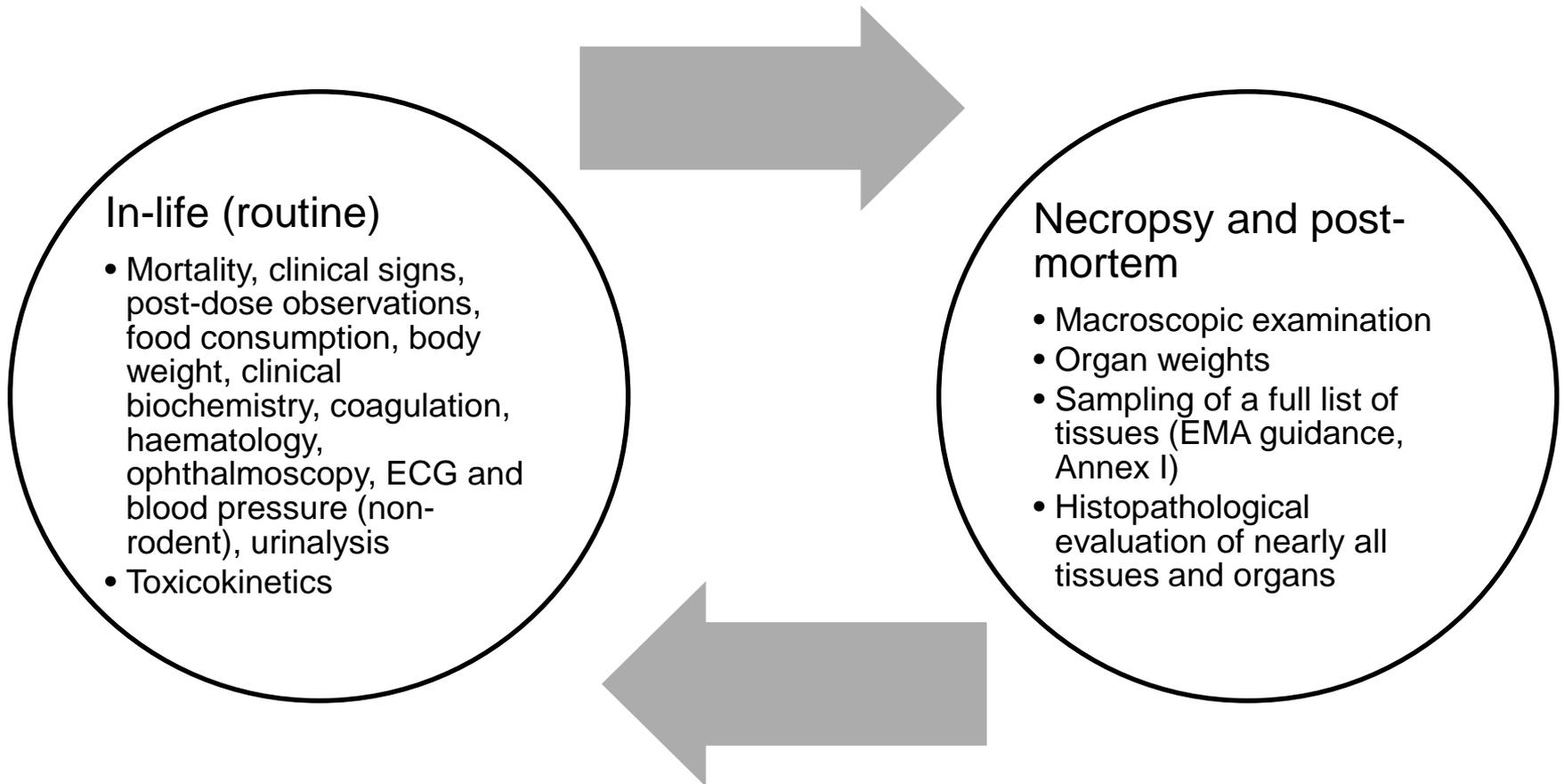
Principal Aims (2)



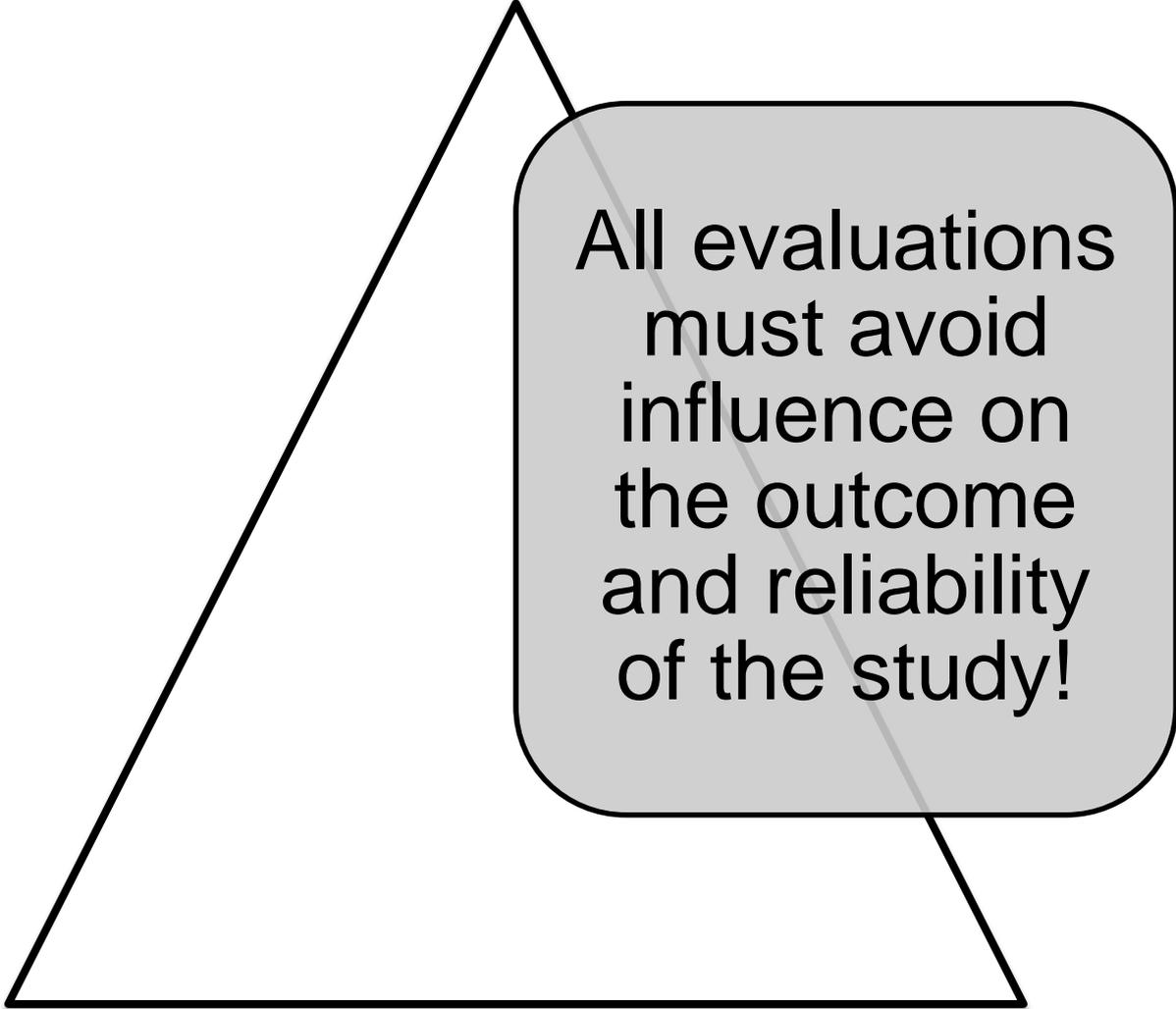
Treatment duration



- 1 day up to 6 months (rodent) or 9 months (non-rodent)
- Duration of treatment in chronic toxicity studies see ICH S4



In life observations

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All evaluations must avoid influence on the outcome and reliability of the study!

Macroscopic examination

- External features and orifices
- Remove cranial roof
- Observation of brain, pituitary gland, cranial nerves
- Ventral mid-line incision
- Expose neck, associated tissues, thoracic, abdominal pelvic cavities and their viscera
- Examine *in situ*

Necropsy and post-mortem (2)



Organ weights

- Selected organs (e.g. adrenal, brain, testes, ovaries, epididymes, heart, kidney, liver, lungs with mainstem bronchi, spleen, thymus, thyroid with parathyroids, uterus with cervix)
- Examine external and cut surfaces

Sampling of a full list of tissues (EMA guidance)

Preserve tissues and prepare wax blocks where appropriate

Histopathological evaluation



Of nearly all tissues and organs	Rodents (more animals/group)	Non-rodents (fewer animals/group)
<ul style="list-style-type: none">• Total number of animals/sex/group dependent on study duration	<ul style="list-style-type: none">• Initially, all controls and high-dose animals• Subsequently, read-down of the groups for any target organs identified• Additional histopathology will be included as necessary• Routine stains are HE (Haematoxylin-Eosin)• Additional stains may be required to further evaluate potential effects	<ul style="list-style-type: none">• Each animal in the study will be examined

Histopathological evaluation (2)



Which endpoints to include will determine how to process tissues

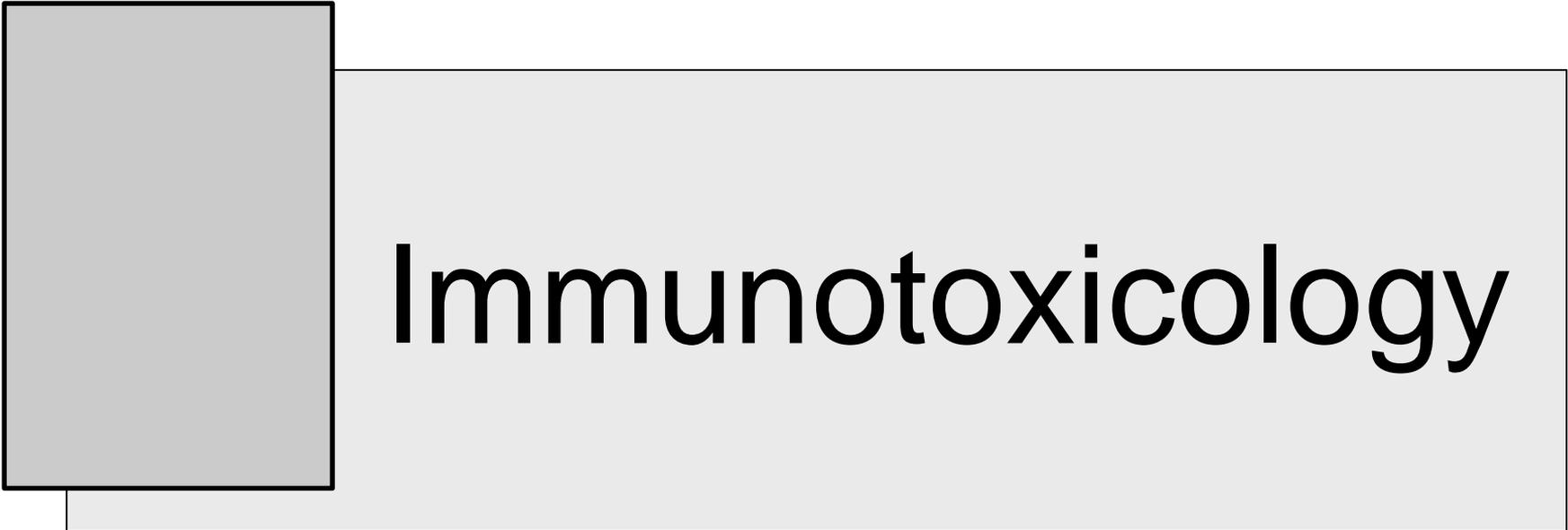
- Decisions need to be made prospectively
- For technical reasons, follow-up investigations may not always be possible from an initial study
- Additional studies may be required

Electron microscopy may be considered in follow-up studies

Additional investigations



- “If immunologic effects are anticipated with the compound or if there is evidence of immunologic activation or inhibition in repeated dose toxicity studies, immunotoxicity of the compound should be explored in accordance with the *Guideline on Immunotoxicity of Human Pharmaceuticals* (CPMP/ICH/SWP/167235/2004; ICH S8).”
- Neurotoxicity
 - FOBs can be included – i.e. following repeated dosing
- Additional sub-sets of animals
 - Interim and recovery animals to be added



Immunotoxicology

- Objectives

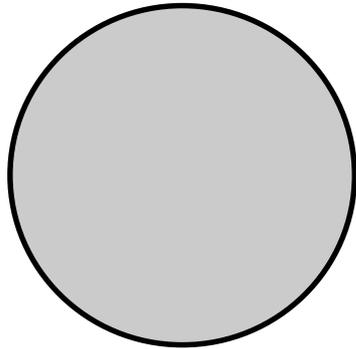
1. “...recommendations on nonclinical testing approaches to identify compounds which have the potential to be immunotoxic.
2. ... a weight-of-evidence decision making approach for immunotoxicity testing.

Immunotoxicity is, for the purpose of this guideline, defined as unintended immunosuppression or enhancement.

Drug-induced hypersensitivity and autoimmunity are excluded.”

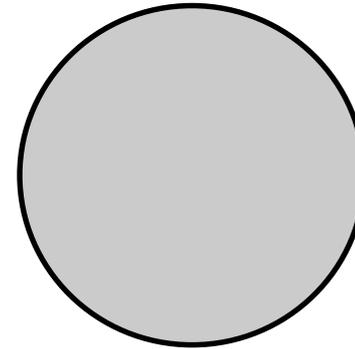
- Note: animals, in general, are poor predictors of human immunotoxicity

Immunotoxicity ICH S8 (2)

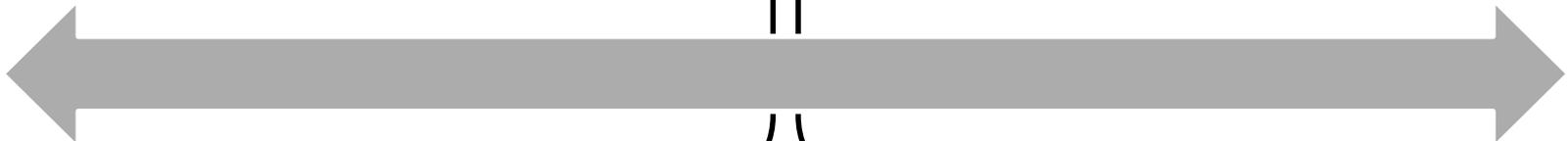


Guideline applies

- To small molecules
- Not to biologics
 - Refers back to ICH S6(R1)



No standard approaches but general recommendations



General principle (ICH S8)



All new human pharmaceuticals should be evaluated for the potential to produce immunotoxicity;

Whether additional immunotoxicity studies are appropriate should be determined by a weight of evidence review of factor(s) [...].

Methods include standard toxicity studies (STS) and additional immunotoxicity studies conducted **as appropriate**.

Factors to be considered (ICH S8)



(1) findings from standard toxicology studies

(2) the pharmacological properties of the drug

(3) the intended patient population (immunocompromised?)

(4) structural similarities to known immunomodulators

(5) the disposition of the drug

(6) clinical information

Methods to evaluate immunotoxicity



Standard toxicology studies

- Haematology: Total and absolute differential leukocyte counts
- Clinical chemistry: Globulin levels and Albumin/Globulin ratio
- Macropathology at necropsy: Lymphoid organs/tissues
- Organ weights: Thymus/spleen (optional: lymph nodes)
- Histopathology: Lymphoid tissues incl. bone marrow (if signal from other endpoints)

Signs from standard toxicology studies



Haematological changes

- Leukocytopenia/leukocytosis
- Granulocytopenia/granulocytosis
- Lymphocytopenia/lymphocytosis
- (Thrombocytopenia/thrombocytosis)

Alterations in immune system organ weights and/or histology

- Thymus spleen, lymph nodes, bone marrow

Signs from standard toxicology studies (2)



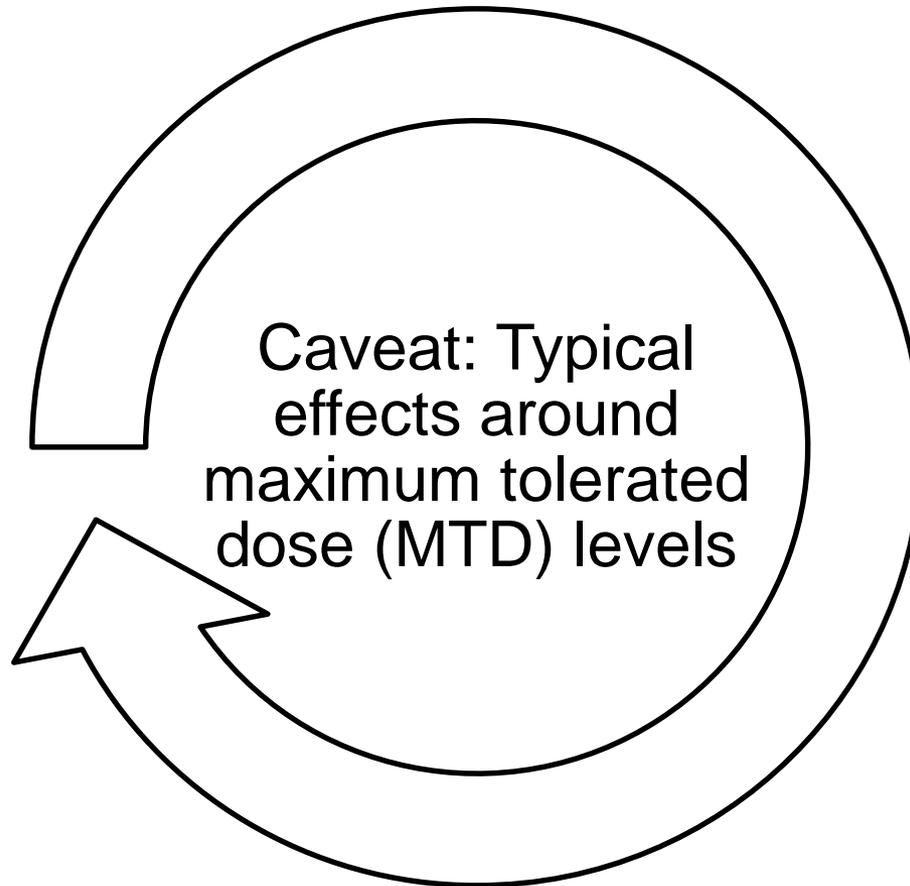
Changes in serum globulins

- Occurring without plausible explanation
 - e.g. concomitant effects on liver/kidney
- May indicate changes in serum immunoglobulins

Increased incidence of infections or tumours

- In the absence of plausible causes
 - Such as genetic toxicity, hormonal effects, liver/enzyme induction

Signs from standard toxicology studies (3)



- Secondary to unspecific stress
- Overload of the system with high drug amounts
- Associated with exaggerated pharmacological or toxicological effects

Typical stress-related findings



Thymic involution/decreases in thymus weight

Reduced cortical cellularity in thymus

Changes in spleen and lymph node cellularity

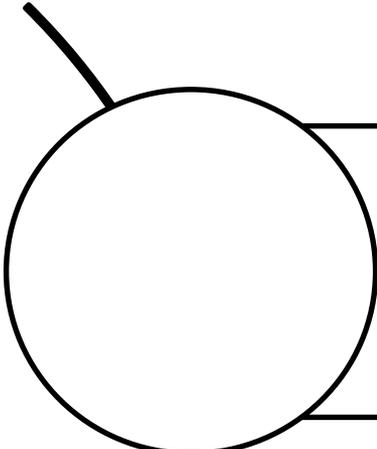
Increases in circulating neutrophils

Decreases in circulating lymphocytes

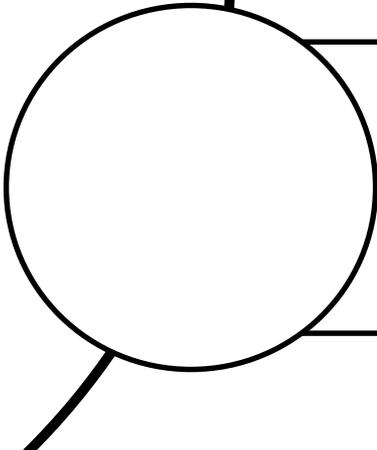
Increase in adrenal gland weights

Adrenal cortical hypertrophy/hyperplasia

Typical stress-related findings (2)

A diagram of a speech bubble, consisting of a circle with a tail pointing towards the top left. The circle is connected to a horizontal rectangular box containing text.

These observations typically do not indicate a specific immunotoxic activity of the test item if they occur around or above an MTD

A diagram of a speech bubble, consisting of a circle with a tail pointing towards the bottom left. The circle is connected to a horizontal rectangular box containing text.

More likely mediated via a stress-induced unspecific increase in systemic corticosteroide levels and other mediators

Additional immunotoxicity studies



- Required if there is cause for concern
- Type of studies dependent on
 - Nature of the findings
 - Compound class
- Usually required before large-scale clinical trials
 - However, timing depends on level of concern in target patient population (immunocompromised?)
- Possible outcomes
 - No risk identified
 - Risk identified but data insufficient for making a risk-benefit decision – will require more studies
 - Established risk of immunotoxicity – further steps depend on risk-benefit ratio

Immunotoxicity *in vivo* study



- Generally accepted design
- 28 day study in rodents
- In general both sexes (rodents)
 - One sex acceptable in non-human primates (NHP)
- Species, strain, dose, duration and route of administration used in additional studies should be consistent with the standard toxicology studies
- Contradicts further guidance on dose selection
 - High dose should be
 - > NOAEL in the standard toxicology study
 - But below a dose level associated with changes secondary to general stress
 - Multiple dose levels incl. a NOAEL for immunotoxicity

Possible assays



- TDAR: T-cell dependent anti-body response
- Uses a recognised T-cell dependent antigen e.g.
 - KLH (keyhole limpet haemocyanin)
 - SRBC (sheep red blood cell)
- Results in a robust anti-body response
- Antibody measurement can be via ELISA or other appropriate immunoassays
- Response depends on species (non-human primate, mouse, rat)
- Serial blood collection might be needed (non-human primate)

Possible assays (2)



- Immunophenotyping
 - Principle: identification and/or enumeration of leukocyte subsets using antibodies
 - Absolute and relative figures
 - Flow cytometry
 - Not functional (for enumeration)
 - Immunohistochemistry
 - Can be added to standard studies as additional endpoint
 - Tissues can also be analysed retrospectively if cause of concern identified later in development
 - But: some lymphocyte markers are sensitive to formalin fixation (standard fixative)
 - Require flash frozen tissues or specific fixatives
 - Compartmental changes of cell types can be diagnosed
 - Quantification more difficult

Possible assays (3)



- Natural killer cell assays
 - Follow-up to alterations diagnosed at immunophenotyping
 - Increased viral infection rates in standard toxicology studies
 - Others
 - Ex vivo assay
 - Spleen or blood sampled from treated animals
 - Cell counts and function can be evaluated
 - NK function evaluated by determining cytolytic activity against target tumour cells
- Other assays
 - Host resistant studies
 - Macrophage/neutrophil function
 - Cell mediated immunity (not as well established)

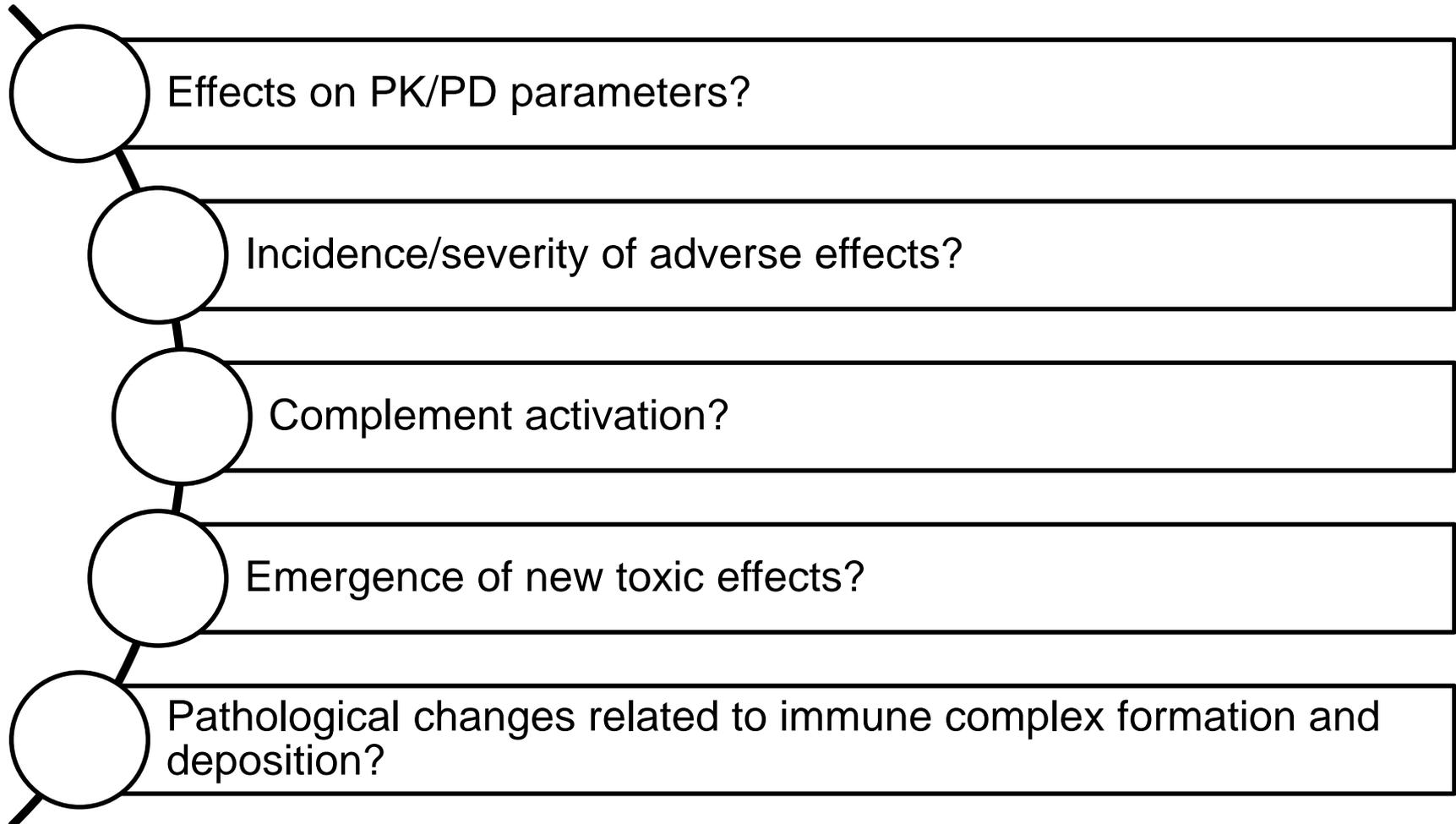
Immunogenicity in animal models frequent

Formation of antibodies – neutralising/non-neutralising

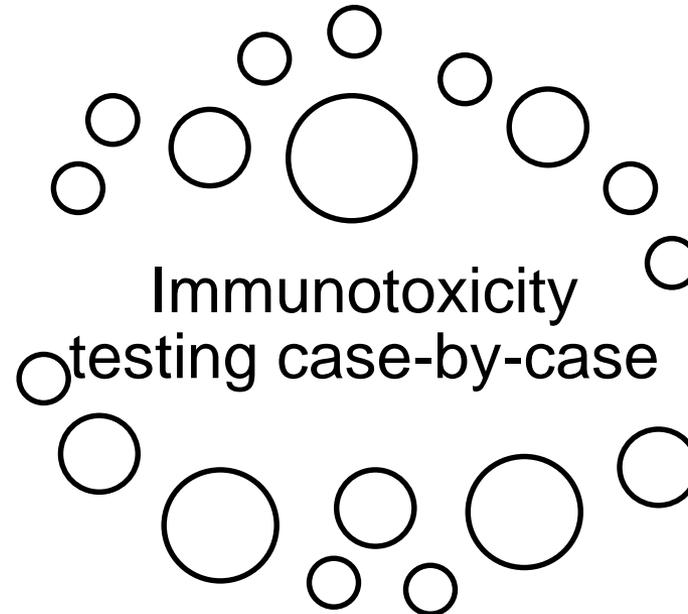
Not predictive for antibody-formation in humans

However, important for interpretation of findings in the animal studies

Correlation of appearance with pharmacological/ toxicological effects (ICH S6 (R1))



If the interpretation of the data from the safety study is not compromised by these issues, then no special significance should be ascribed to the antibody response



- Many biotechnology-derived pharmaceuticals intended to stimulate or suppress the immune system
- May affect not only humoral but also cell-mediated immunity

Immunomodulatory biologics



- Typical indications like cancer and auto-immune diseases
- Adverse reactions in humans may include
 - Serious infections
 - Malignancy
 - Cytokine release syndrome
 - Anaphylaxis
 - Hypersensitivity
 - Immunogenicity (in humans)
 - Autoimmunity

Refer to Sathish JG et al. Nat Rev Drug Discov. 2013 Apr;12(4):306-24



Take home messages

Take home messages



- Absence of evidence is not evidence of absence!
- Expect the unexpected!
- The immune system may be a target organ of toxicity for any type of compound
- Weight of evidence approach
- Careful case by case assessments
- Learn from clinical experience!
- Integrated and interdisciplinary approach required to translate data across species

PreClinical Safety (PCS) Consultants Ltd

Nauenstrasse 63A

CH-4052 Basel

Switzerland

Website: www.pcsconsultants.com

Dr. med. vet. Stephanie Plassmann

Board Certified Specialist in Veterinary Pharmacology and Toxicology

Tel: +49 8133 908896

e-mail: stephanie.plassmann@pcsconsultants.com