Consideration Points for Nonclinical Safety Testing of Biotherapeutics

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Highlights

- Impact of large molecule on safety assessment
- Establish species relevance for testing animal models
- Consideration points in biotherapeutics' preclinical safety assessment
- Studies/data not typically relevant for biotherapeutics
- Current preclinical capability in China to support biotherapeutic development
- Biologic development-related regional regulatory landscape

Types of Biotherapeutic Products*

Biopharmaceuticls are a diverse class of human therapeutics generally produced in characterized cells

Center for Drug Evaluation and Research	Center for Biologics Evaluation and Research
Hormones	*Blood and tissue product*
Cytokines, growth factors	*Vaccines*
Antagonists/ inhibitors	*Gene-transfer products*
Monoclonal antibodies and related products	*Cell-based therapies*
Modified human proteins	*Tissue-engineered products*

The goals of preclinical safety assessment for biotherapeutics

Aims of nonclinical safety evaluation

- Identify target organs for potential toxicity
- Determine reversibility if toxicity observed
- Help to determine clinical starting dose, maximum dose and dose escalation scheme for Phase I
- Identify potential biomarker/parameters for clinical safety monitoring if there is any safety signal
- Provide data for safety information on the label (communicate risk)

In comparison to small molecules, biotherapeutics have:

Features of Biotherapeutic	Impact on safety assessment
Large molecules, proteins	
■ Limited ability to cross biological membranes	■ Genotoxicity assays generally not relevant (exceptions - cytotoxic conjugate)
■ Degraded into amino acids (lack of reactive metabolites)	■ Less frequent dosing but long recovery/washout period
■ Long half-life	
Specific human target	
■ Human target absent in most- if not all - animal species	■ Potential issue of species relevance
Target-related biological activity	■ Rare off-target toxicity
■ Toxicity mostly related to pharmacological effects	■ "case by case" safety assessment vs. "check box"

Consideration point 1:
Establish species relevance for testing animal models

- **The most important factor in safety evaluation of a biologic is selection of a relevant species**
  - **Species expresses desired target or epitope**
  - **Biologic exerts expected pharmacologic effects**
  - **Tissue distribution of target is similar between human and test species**
- **Identify relevant species based on scientific rationale**
  - **Often nonhuman primate is only relevant species**
  - **Occasionally, when no relevant species - alternative approaches:**
    - Surrogate antibody
    - Transgenic model
  - **Conducing tox studies in non-relevant species is to be discouraged**
    - Unreliable safety data (no toxicity)
    - No off-target effects expected
  - **If no relevant species or alternative in vivo model, in vitro safety package may be considered for some indications**
    - Will result in very slow clinical dose escalation

Demonstrate toxicology species is pharmacologically relevant

Practical approach for species relevance determination

- Sequence homology as compared to human: %
- Distribution of target as compared to human: expression ratio and intensity
- Binding affinity as compared to human : nM
- Functional assay as compared to human: e.g. % inhibition (Expression does not necessarily mean function)

Consideration point 2: Immunogenicity evaluation (1)

- **Human proteins often immunogenic in animal species**
- **Immunogenicity in animals is not predictive for humans**
  - **Anti-drug antibodies (ADA) should be assessed for adequate interpretation of tox studies**
    - Increase clearance and decrease exposure
    - Neutralization of pharmacologic effects
    - Cross-reactive with endogenous protein limiting function – altered function can lead to toxicity

Consideration point 2: Immunogenicity evaluation (2)

- When ADAs are detected
  - The effect on the study results should be addressed: PK, PD, and toxicity
- Immunogenicity issues in toxicity studies can often be addressed through study design modifications
  - Immunogenicity often inversely proportional to dose
  - s.c. route more immunogenic than i.v. route
  - Increase group size to ensure enough animals with adequate exposure

Consideration point 3: Immunomodulation and immunotoxicity

- **Immunomodulation - intended**
  - **Intended PD effect on immune system**
- **Immunotoxicity - unintended**
  - **Unintended impairment of any component of immune function**
  - **Major risks**
    - Acute reaction, cytokine storm, chronic immunosuppression >> opportunistic infections and cancer
  - **ICH S6 principles should be applied for safety assessment**
    - May require screening studies and/or mechanistic studies

Consideration point 4: Tissue Cross-reactivity

Tissue cross-reactivity (TCR) study

- Required prior to entry of mAbs into Phase I
- The tested tissues come from human and animal donors
- IHC in a broad panel of frozen tissues
- Identify off-target binding and target expression in non-disease tissue
- Not specified but normally conducted under GLP
- Not required for justification of tox species relevance
- Differences in staining pattern between human and animal does not preclude using species for tox studies
- IHC can be useful for interpretation of toxicology studies

Consideration point 5: General Tox Study Design (1)

Route of administration:

- Biologics usually not administered orally
- Mimic clinical route
- In some cases, IV toxicology studies may support clinical studies with subcutaneous administration with a local tolerance test
- Other routes may be used – IM, intra-articular, intrathecal

Consideration point 5: General Tox Study Design (2)

Dose selection

- **Maximum tolerated doses may not be achieved due to limited toxicity**
  - Not necessary to elevate the dose to identify MTD
- **Dose multiples may be scientifically justified using several approaches**
  - Target binding/saturation
  - Maximum pharmacologically active dose
  - Highest anticipated clinical dose/exposure
- **Appropriate high dose multiples typically range from 10-25x**
- **Distribution of large biologics (≥ 100 kDa) is limited to extracellular space**
  - Dose extrapolation from tox studies is on mg/kg basis

Consideration point 5: General Tox Study Design (3)

Frequency of Dosing

- **Biologics typically have long half-lives (1-2 weeks), except for Fab fragments have shorter half-lives (hours)**
  - Infrequent dosing but long recovery period
- **Administered at least as frequently as intended clinic schedule**
  - Differences in PK between human and tox species should be considered
- **Increasing frequency may help to reach higher exposure**
- **Frequency may be increased to overcome a clearing ADA response**

Consideration point 5: General Tox Study Design (4)

Duration

- Typical durations: 1/3/6 months
- Longer than 6 months duration generally not required (ICH S6)
- Single-dose and range finding studies are of limited value

Recovery period

- Examine reversibility of adverse effects
- Duration based on half-life

Consideration point 6: Developmental and Reproductive Toxicity (DART) Studies

- Standard DART studies in rodent/rabbit may be not relevant
- Reproductive toxicity studies should be conducted in relevant species only, and in accordance with the principles outlined in ICH S5(R2)
- Studies can be obviated in some specific cases
  - Extensive public information for a particular class of compounds (e.g. interferons)
- Discussion with the regulatory agencies is still needed

Special consideration 7: Placental transfer

- Large proteins (>5,000 Da) do not cross the placenta by simple diffusion
  - Recombinant receptor proteins
  - Fab fragments demonstrate minimal placental transfer
- Full IgG antibodies are actively transported across the placenta
  - Species specific FcRn-mediated transport determines fetal exposure of some antibodies (mAbs and Fc-fusion protein)
    - Primate placental transfer occurs after organogenesis
    - Placental transfer occurs throughout gestation in rodents
  - IgG isotype effects placental transfer (IgG1>IgG4>IgG3>IgG2)

Special consideration 8: Carcinogenicity

- **Requirement for carcinogenicity studies a major unresolved issue of the ICH S6 Addendum process**
  - **2-year rodent bioassays generally considered inappropriate for biotherapeutics**
    - Species relevance
    - Off-target effects are rare
    - No metabolites
- **Carcinogenicity risk assessment**
  - **Package assembled by sponsor to address the risk**
    - The nature of target/ligand (pharmacology)
    - The target population
    - Signals from toxicology studies
    - Carcinogenic potential of similar compounds
    - Additional in vitro/ in vivo studies as available

Special considerations for biotherapeutics (1): Genetic toxicity

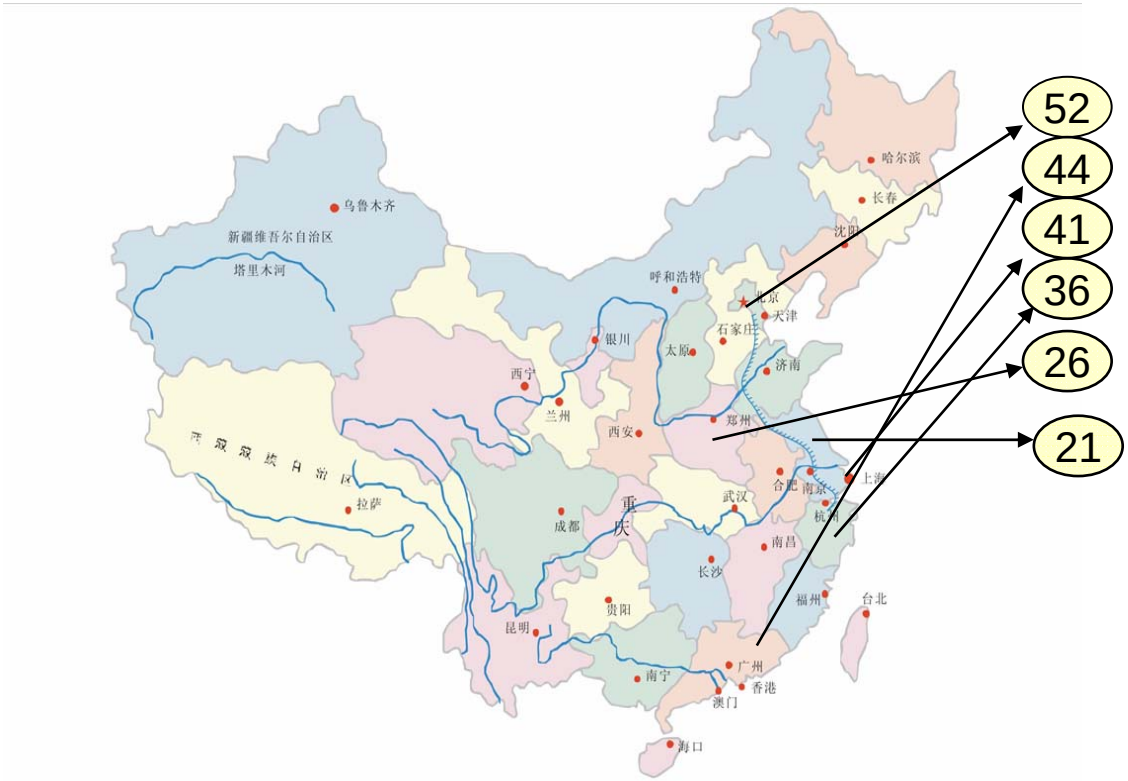
- **Genetic toxicity tests generally not performed with biotherapeutics**
  - **Interaction with genetic material not expected**
  - **No entry into nucleus**
  - **Situations that might need gene tox studies**
    - Impurities
    - Genotoxic conjugates
    - Molecules expected to interact with genetic material

Special considerations for biotherapeutics (2): Safety pharmacology

- Approached on case-by-case basis
- Generally, stand alone safety pharmacology studies are not performed
  - Large peptides and proteins cannot readily penetrate cell membrane
  - Safety pharmacology endpoints may be incorporated into the general toxicology studies (clinical observations, ECG, body temp)

Where the main force of biotech companies in China?

- According to the report on the web of SFDA, 482 biotech companies are recorded.
- The biotech companies are located in 29 provinces or cities all over China. 35.9% of them located in the economy developed districts such as Beijing, Shanghai, Guangzhou Province, Jiangsu Province etc.



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Current biopharmaceutical development in China

- By the end of 2009, total 13 mAb approved by sFDA
- Among them, 7: imported (most homologus); 6: domestic (3鼠源型, 1嵌和型, and 2个人源化单抗)

The response to “sanofi 2011 Investigation Questionnaire”

- The bio-pharmacy questionnaire was sent to 40 CROs all over the country at the beginning of May 2011
- We received 12/40 of the replies from them
- 10/12 have the safety evaluate experience for biologics
- 9/10 have the certificate and experimental condition to conduct preclinical safety assessment with monkeys
- Capable to conduct all required nonclinical safety assessment for biologic development

Current regulatory landscape for biologic development in Asia Pacific

- China: Translated and started to implement ICH S-series guideline including S6
- Japan PMDA and KFDA: Claimed to follow ICH guideline completely
- Taiwan and Hong Kong districts: Claimed to follow ICH guideline completely

Thus, the IND package generated anywhere becomes more internationally portable/submitable.

Questions and Answers