Preclinical safety test strategy for New Biological Entities (NBEs)

Regulatory toxicology workshop – München 2005
Test strategies for NBEs

General principles

• Develop strategy on a case by case basis

• Close interaction with regulatory authorities

• Science driven rather than tick box approach
Test strategies for NBEs

Primary goals

• Idenfiy initial safe dose and subsequent dose escalation schemes in humans

• Identify potential target organs for toxicity and reversibility

• Identify safety parameters for clinical monitoring
## Test strategies for NBEs

### Comparison of NCEs and NBEs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NCEs</th>
<th>NBEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main route of administration</td>
<td>oral</td>
<td>i.v. s.c</td>
</tr>
<tr>
<td>Metabolism</td>
<td>metabolized</td>
<td>catabolized</td>
</tr>
<tr>
<td>Metabolites</td>
<td>could be toxic</td>
<td>no metabolites</td>
</tr>
<tr>
<td>Species limitations</td>
<td>2 species</td>
<td>one may be acceptable</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>rarely</td>
<td>very frequently</td>
</tr>
<tr>
<td>Impurity profile</td>
<td>small molecules</td>
<td>DNA, Viruses, Proteins</td>
</tr>
<tr>
<td>Drug/Drug interaction</td>
<td>potential</td>
<td>rarely</td>
</tr>
<tr>
<td>Toxicity</td>
<td>general tox</td>
<td>can be highly specific</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(target related toxicity)</td>
</tr>
</tbody>
</table>
Test strategies for NBES

Range of biotechnology-derived pharmaceuticals

- Hormones: FSH, LH, Insulin
- Growth factors: NGF,
- Cytokines: Interferons, Interleukins
- Monoclonal antibodies: Murine, chimeric, humanized
- Vaccines: anti-cancer vaccines
- Nucleic acid-based products: Gene therapy
- Others
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Challenges

- Nature of the therapeutic product
- Find relevant animal species
- Design of study
  (dose, exposure levels, endpoints to be monitored)
- Immunogenicity
  (against therapeutic product and modulation of immune status)
Test strategies for NBEs

Challenges (cont.)

• Assessment of toxicity to reproduction

• Assessment of genotoxic potential

• Assessment of carcinogenic potential
Test strategies for NBEs

Species selection

- A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope.
- Relevant animal species for testing monoclonal antibodies are those that express the desired epitope and demonstrate a similar tissue cross-reactivity profile as for human tissues.
- Species which due not express the desired epitope may still be of some relevance if comparable unintentional tissue cross-reactivity to humans is demonstrated.
Test strategies for NBES

Species selection (cont.)

- Normally two relevant species
- One species sufficient if only one relevant species can be identified or where the biological activity is well understood
- Toxicity studies in non-relevant species are discouraged

What can be done when no relevant species exists?

- Use of transgenic animals
- Use of homologous protein in rodent model
Test strategies for NBEs

Administration/dose selection

- Route and frequency of administration should be as close as possible to that proposed for clinical use
- Frequency may be increased compared to clinical schedule to compensate for faster clearance rates in animals
- Dose-response relationship should be established
- Toxic dose and NOAEL should be determined
- Dose levels should at least span therapeutic dose range with regard to AUC
- Multiples of human dose necessary to determine safety margins vary with class of biotechnology-derived pharmaceuticals, patient population and indication
Test strategies for NBEs

Immunogenicity

- Correlate immunogenicity with pharmacological and toxicological response including pharmacokinetics
- Characterize antibodies (issue: assay interference with high monoclonal antibody [MoAb] levels in serum)

Sensitivity of immunoassay <1µg/ml in serum

<table>
<thead>
<tr>
<th>Type of Ab</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding</td>
<td>no effect</td>
</tr>
<tr>
<td>Clearing</td>
<td>clearance↑ and t1/2↓</td>
</tr>
<tr>
<td>Neutralising</td>
<td>pharmacological effect ↓</td>
</tr>
<tr>
<td>Sustaining</td>
<td>AUC, Cmax and t1/2↑</td>
</tr>
<tr>
<td>Cross reactive Ab</td>
<td>Ab against endogenous protein (erythropoietin; Ab against endogenous erythropoietin → pure red cell aplasia)</td>
</tr>
</tbody>
</table>
Test strategies for NBEs

Immunogenicity (cont.)

- Route of administration and substance characteristics can influence immunogenicity (s.c > i.v.)
- Determine incidence and titer of antibody formation, continue dosing of animals which have no Ab or Ab do impair pharmacological activity
- Compare individual values with predose values (longitudinal comparison more important than statistical differences from control)

Goal

- Minimize anti drug Ab response
- Appropriate species selection
- Surrogate model
- Ab engineering process should select Ab based on ability to cross react with monkey test species, even if Ab has lower affinity (SOT meeting 2005)
Test strategies for NBEs

Assessment of reproductive toxicity

- Extent of evaluation will depend on the clinical indication, patient population, disease severity, availability of alternative therapies, availability of a relevant model, immunogenicity issues in the animal model

- Standard rat and rabbit reproduction toxicity studies may be problematic

- Primate model often only relevant model (e.g. interferons)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>similarity to humans in response to human teratogens</td>
<td>high spontaneous abortion rate</td>
</tr>
<tr>
<td>menstrual cycle</td>
<td>high animal number (8/12/group)</td>
</tr>
<tr>
<td>placental morphology</td>
<td>duration of study 1-2 years</td>
</tr>
<tr>
<td></td>
<td>expensive</td>
</tr>
</tbody>
</table>
Test strategies for NBEs

Assessment of reproductive toxicity (cont.)

• Ab can cross placenta certain Ab with prolonged immunological effects should be addressed in a study design modified to assess immune function of the neonate

• Use of homologous proteins
• Use of transgenic mouse model

Goal: optimise predictive value of studies
Test strategies for NBES

Assessment of genotoxicity

• Generally not required

  but consider if

• Anorganic linker molecules are used
• Toxin conjugates
Assessment of carcinogenic potential

• Conventional approach rarely appropriate for biotechnology products
• For some products e.g. growth factors, immunosuppressive monoclonal antibodies a potential hazard must be assumed
• What can be done?
• Ability of the product to stimulate growth of normal or malignant human cells expressing the receptor/epitope should be determined in vitro
• If in vitro data give reason for concern further in vivo studies may be needed
• Incorporation of sensitive indices of cellular proliferation in long term repeated dose toxicity studies may provide useful information
Test strategies for NBEs

Comparability of products (EMEA, 2003)

- Comparability exercise should be carried out when change is introduced either during development, i.e. after critical studies (demonstration of product consistency, stability studies, pre-clinical studies, pivotal phase II/III studies) have been initiated or after marketing authorisation has been granted.

Factors to be taken into consideration

- Complexity of the molecular structure
- Type of changes introduced in the manufacturing process
- Impact on quality, safety and efficacy (purity, identity, stability, potency)

Consequences

- Bridging studies may be necessary to address underlying issues relating to pre-clinical pharmacology/toxicology and clinical safety/efficacy.

(FDA statement on SOT 2005, clinical study was placed on hold due to lack of comparability)
Test strategies for NBEs

Examples (Data from FDA data base)

1. Conventional approach similar to NCE

Erythropoetin Darbepoetin alfa (Aranesp®)

- rHu EPO responsive in rat, dog, monkey
- Conventional approach in toxicology testing
- Single dose study in rats and dogs
- 1,3 and 6 month toxicity studies with recovery in rats and dogs
- Full reproductive toxicology package
- Safety pharmacology
Test strategies for NBES

Examples (Data from FDA data base)

2. Mixed approach, conventional and surrogate, KO, transgenic

Infliximab (Remicade®) – Chimeric (human/murine) IgG1 Ab for use in Crohn’s patients

Pharmacology

- In vitro cross reactivity with human fibroblasts, endothelial cells, epithelial cells, PMC
- Surrogate anti-mouse TNFalpha antibody cV1q in transgenic murine colitis model tested
- Tg models expressing constitutively human TNFalpha (e.g. Tg211) were used for pharmacology testing
- Human tissue cross reactivity revealed only binding to tissues which express TNFalpha
- No binding in rodents, rabbits, marmoset, baboons, rhesus, cynomolgus only very weakly in dogs (dogs developed hypersensitivity syndrome after single dose, therefore not suitable for tox species)
- Chimpanzee is the only species other than humans whose TNFalpha binds to cA2
Test strategies for NBEs

Examples (Data from FDA data base)

2. Mixed approach, conventional and surrogate, KO, transgenic

Toxicology

• Repeat dose toxicity studies limited to a few in vivo tests in chimpanzees (hematology, clinical pathology, kinetics, immunogenicity)

• Developmental toxicity study in mice using surrogate Mab against mouse TNF-alpha-; Ab crossed placenta and kinetic studies proved exposure of pups- no evidence of impairment of reproductive function, embryotoxicity or teratogenicity

• Additionally a knock-out mouse for TNF-alpha was generated and this strain did not show any evidence of impairment of reproduction

• Complete battery of genotoxicity studies performed (2 in vitro plus 1 in vivo)

• No carcinogenicity studies performed
Test strategies for NBEs

Examples (Data from FDA data base)

2. Mixed approach, conventional, surrogate, KO, transgenic

Toxicology (cont.)

Conclusions

• Limited toxicity testing in chimpanzees (only relevant animal model)
• Data from genetically modified animals (i.e. transgenic and KO models) and from studies with the surrogate Ab cV1q against mouse TNF-alpha have provided some reassurance regarding reproductive and carcinogenic potential
• Due to the lack of relevant animal models, the absence of more detailed toxicity data with infliximab was considered acceptable
Test strategies for NBEs

Examples (Data from FDA data base)

3. Mixed approach, conventional and surrogate model
Efalizumab (Raptiva\textsuperscript{R}) – treatment for Psoriasis
Efalizumab recognizes only human and chimpanzee CD11a
• Binding to lymphocyte function antigen-1 blocks binding to intercellular adhesion molecule-1 – inhibition of T-lymphocyte function
• muM17 appropriate surrogate molecule for efalizumab

Toxicology
• General toxicity studies in chimpanzees (only clinical) and TSG-p53 mice
• Duration of repeat dose toxicity studies of up to 6 months
• Safety pharmacology endpoints in tox studies
Test strategies for NBEs

Examples (Data from FDA data base)

Toxicology (cont.)

- Reproductive toxicology studies in CD1 mice (embryo-fetal toxicity study and peri-and postnatal study, immunology endpoints measured in dams and offsprings
- Assessment of immunotoxicology in separate studies and in long-term repeat dose study in chimpanzee which included lymph node biopsies and ability to mount antibody response to tetanus toxoid, reversibility could be shown, no evidence for increase in infections
- No genotoxicity and carcinogenicity studies

Conclusions

- Limited toxicity testing in Chimpanzees (only relevant animal model)
- Data with surrogate molecule in mice against murine CD11a considered to be appropriate
Test strategies for NBEs

Summary

• Toxicity testing using conventional study designs often not appropriate
• Importance of selecting relevant animal model, dose/exposure and endpoints
• Homologous proteins/transgenic models may provide useful information for safety evaluation
• Limitations still exist for the pre-clinical assessment of reproductive toxicology and carcinogenicity for some biotechnology products
• Case by case decision on strategy still best advice
Test strategies for NBEs

- Keliximab is a human-cynomolgus monkey chimeric monoclonal antibody which is specific for human and Chimpanzee
- CD4 human knock in and CD4 mouse knock out
- Human CD4 is functionally active in mice
- Model was extensively characterized
  - Longevity
  - Reproductive performance
  - Background pathologies
- Mouse model used for repeated dose, reproductive and genotoxicity studies
- Intensive immunotoxicity studies to investigate effect on host resistance to infections (Candida albicans, Pneumocystis carinii) and on immunosurveillance to neoplasia