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Development of Humanized Monoclonal Antibodies

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Introduction

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- **Biotechnology is one of the most emerging technologies of the 21st Century**
- **Transformation of scientific discoveries into applicable human drugs is the most challenging task and requires expert knowledge and enthusiasm**

Biological Products

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- **Vaccines**
  - Therapeutic (cancer vaccine)
  - Preventative (childhood vaccines)
- **Therapeutic proteins**
  - Monoclonal antibodies (anti-TNF- Infliximab, Enbrel)
  - Interferons and interleukins (IFN-alpha)
  - Growth factors (erythropoietin, G-CSF)
  - Enzymes (asparaginase, rasburicase)
  - Thrombolytics (streptokinase)

Monoclonal Antibodies - Background

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- 1896 Paul Ehrlich (1908 Nobel Prize) – side chain theory
- 1936 Heidelberger and Kendall – Isolated antibodies
- 1959 Edelman and Porter (1972 Nobel Prize) – antibody structure
- 1975 Kohler and Milstein (1984 Nobel Prize) – hybridoma technique – B cells fused with tumor cells – production of any type of mAbs

Classes of Monoclonal Antibodies - Currently Licensed

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- **Antitumoural (cytotoxic) antibodies**
  - **Avastin® (bevacizumab), Erbitux® (cetuximab), Herceptin® (trastuzumab), MabCampath® (alemtuzumab), MabThera® (rituximab).**
- **Immunomodulators**
  - **Humira® (adalimumab), Remicade® (infliximab), Raptiva® (efalizumab), Simulect®; (basiliximab), Zenapax® (daclizumab).**
- **- Pathogen-specific mAbs**
  - **Synagis® (palivizumab)**
- **Others**
  - **ReoPro® (abciximab), CEA-Scan® (arcitumomab), Leukoscan® (sulesomab)**

Monoclonal Antibodies
- highly complex molecules

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- **- High molecular weight**
- **- Complexity**
  - (primary / secondary / tertiary / quarternay structure;
    post-translational modifications)
- **- Heterogeneity (drug substance, drug product)**
- **- Process- and product-related impurities**
- **- Low stability of drug substance / drug product**
- **- Species specificity**
- **- Immunogenicity**

Issues with Monoclonal Antibodies

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- **Quality**
  - **- impurities**
  - **- batch inconsistency**
  - **- contaminants**
  - **- microheterogeneity**
  - **- fragments**
- **Preclinical**
  - **- tissue cross-reactivity?**
  - **- toxicity?**
  - **- immunotoxicity?**

Central Apect:
mAbs are species-specific.

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- **A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies)*.**

Non-Clinical considerations

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- **Monoclonal antibodies can cross-react**
  - **cross-reactivity with structurally related epitopes / receptors?**
    e.g., EGFR – HER2
  - **expression of epitope on "non-target" tissues?**
- **Consequence of non-target tissue expression / cross-reactivity?**
- **Monoclonal antibodies can have differential effects at lower doses**
  - **classical approach: Test a multiple of the clinical target dose in the animal model => safety margin**
  - **MAbs: Test a multiple of the clinical target dose in the animal model => safety margin**
  - and **Test also lower doses to be studied in humans**

TG 1412

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1. **TG1412 is a "molecule targeting agent“**
2. **The target is CD28, a molecule on the surface of lymphocytes that is deeply related to immunity and inflammatory reaction**
3. **The agent is a monoclonal antibody (mab) that combines with CD28**
4. **The agent was expected to stimulate lymphocytes and to induce a substance that suppresses inflammation for treating intractable rheumatism and chronic leukemia**
5. **Conventionally, drugs are mainly chemical, but now the trend is shifting to development of biological substances**
6. TeGenero (Germany) developed the agent
7. CD28 homology human – cynomolgus: 100% (extracellular domain)

Lessons from the TGN1412 case

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- Data on downstream effects with TGN1412 in cynomolgus monkey were presented
- Fist-in-man trial was authorized by MHRA (UK)
- TGN1412 for the first human trial in London on 13 March 2006
- PAREXEL, a CRO that conducted the trial in UK
- TGN1412 dose administered was **one five hundredth** of that proved safe in animal tests
- Right after the six volunteers took the agent, they all started to complained:
  - of body ache and difficulty in breathing and fell unconscious
  - Within an hour they were admitted to ICU for multiple organ failure (MOF) and needed to be on ventilator.

Lesions from the TGN1412 case

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- Predictivity of animal data not 100% (estimates: 70-80%)
- Nevertheless non-clinical data of highest importance
- Not all MAbs are that dangerous, still major "drugs of hope"
- Definition of "high-risk" mAbs for which enhanced precautions need to be employed:
  - extended pre-clinical development before human testing
  - sequential inclusion of subjects into phase I first-in-man trial

High-risk Monoclonal Antibodies

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Criterion 1:

- **The mAb employs a new mechanism of action**
  - mAbs interfering with „master switches“ of the immune system
  - Inducers / modulators of pleiotropic cytokines (IFNγ, IFNα, IL-10)

Criterion 2:

- **The mAb addresses a target that lacks appropriate animal models**
  - (sub-) epitopes that are only present in humans
  - No surrogate model exists
  - Interference with signaling pathways with human-specific properties

Criterion 3:

- **The mAb comprises a new type of engineered structural format**
  - Engineered Fc parts
  - Divalent (bispecific) antibodies etc.

Differences Between Traditional Drugs And Biologics

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- Small molecules (drugs)
  - Chemically synthesized
  - Low molecular weight
  - Generally active in many species
  - Generally no immunogenicity
  - Metabolism
  - Toxicity from parent or metabolite

- Biologics
  - From living cells
  - High molecular weight
  - Highly targeted
  - Responsive (relevant) and non-responsive species
  - Immunogenicity
  - Proteolytic degradation
  - Exaggerated pharmacology

Documents Specific to the Preclinical Development of Biologics

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- **ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (1997)**

- **Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997)**

Concepts Unique to Biologics

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- Relevant species
- Tissue cross-reactivity
- Immunogenicity

Relevant Species

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- **"A relevant species is one in which the test material is pharmacologically active due to the expression of a receptor or an epitope (in the case of monoclonal antibodies)."**
- **"Toxicity studies in non-relevant species may be misleading and are discouraged."**

[ICH S6]

Defining the Relevant Species

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- **Functional pharmacology studies**
- **Tissue cross-reactivity to assess binding of monoclonal antibodies (mAbs) to non-human tissue**

Immunogenicity

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- Many biologics elicit an immune response in animals
- Preclinical and clinical impact
  - Neutralize activity (interfere with binding or increase elimination)
  - Sustain activity (decrease elimination)
  - Cross-react with endogenous compounds
- Included as an endpoint in toxicology studies
- Animal findings not necessarily predictive of human

Goals of Preclinical Safety Evaluation

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- Recommend initial safe starting dose and
- safe dose-escalation scheme in humans
- Identify potential target organ(s) of toxicity
- Identify appropriate parameters for clinical monitoring
- Identify "at risk" patient populations

Preclinical Development

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- **Activities of this phase are aimed at generating enough information about safety and disposition of the drug for safety to humans**
  - **Efficacy**
  - **Toxicology**
  - **Safety pharmacology**
  - **Drug Metabolism**

Establish Efficacy

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- **Having established that a group of molecules interact with the biological target in the desired way, it is important to understand whether an effect can be created *In Vivo,* in an appropriate animal model *or against target cellular organisms***
- **Sometimes, it is only possible to establish efficacy against an *In Situ* model using isolated tissues**

Establish Dose Response

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- **Using the biological model, it is important to establish how the effect varies with dose and how a group of compounds might differ in terms of relative potency**

Early Questions?

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- Can the parent drug be measured in biological materials over the right concentration range under GLP standards
- Is the drug stable in biological matrices under normal handling and storage conditions?
- Is the drug stable in the formulations to be used?
- Is the drug metabolized and if so, to what?
- Does the drug cross intestinal epithelium as predicted by Caco-2 cell permeation studies?
- What is the plasma half-life in rats and monkeys following single dose intravenous administration?

Early Questions?

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- Dos the drug bind to plasma proteins?
- Does he drug inhibit any of the drug metabolizing enzymes (CYPs)?
- Is the drug absorbed if given orally? If so, what is it's bioavailability?
- What should be the species for subchronic studies based on metabolism and pharmackinetic considerations?
- At what dose does the drug show limiting toxicity?
- What are the target organs?

Preclinical Studies Needed for a Phase 1 Study

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Study	Biologic	Small Molecules
Pharmacology	**Yes**	**YES**
Safety Pharmacology	Maybe (usually incorporated in general toxicology study)	Yes (usually standard models)
Toxicology (acute/repeat dose)	**Yes** **(1 species acceptable)**	**Yes** (2 species)
Toxicology(acute/repeat dose)	**Yes** (1 species acceptable)	**Yes** (2 species)
Genotoxicity	**Generally Not Required**	**Yes**

Preclinical Studies Needed for a Phase 1 Study

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Study	Biologic	Small Molecules
Tissue cross-reactivity	**Yes** (monoclonal antibodies)	**Not Required**
Local Tolerance	**Yes** (incorporated in toxicology study)	**Yes**

Toxicology Studies Needed for Licensure
Biologics vs. Small Molecules

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Toxicity Testing	BLA Approval	NDA Approval
ReproductiveToxicity	-Relevant species -Embryo-fetal development -Select species closest to human physiology	Performed in at least 2 species, standard models of rat and rabbit
Carcinogenicity	-Interested in effects on cell proliferation, tumor promotion-In vitro and in vivo studies (alternative models)	-Interested in direct interaction with DNA-Performed in standard models (rodent), sometimes alternative models
Immunotoxicity	Interested in systemic exposure of biologic and consequence of anti-product antibodies	Centers on hypersensitivity and modification of immune system as an adverse event

Mutagenicity

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- Assays designed to detect substances that directly interact with DNA and induce gene mutations, chromosome aberrations and/or DNA damage
- Small molecules
  - Standard 3 assays performed: Ames, CHO and Mouse Micronucleus
- Biologics
  - Genotoxicity assays typically not performed
    - Not expected that proteins would interact with DNA
    - Manufacturing involves physical separation as opposed to the introduction of organic chemicals
- Presence of organic impurities or organic linkers might warrant genotoxicity testing

Safety Pharmacology

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- **Safety pharmacology studies asses the potential undesirable pharmacodynamic effects of a substance on physiological functions**
- **<u>Core battery:</u> CNS, cardiovascular and respiratory systems**
- **For highly targeted biologics, safety pharmacology endpoints may be included in toxicology or pharmacodynamic studies**

ADME Studies

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- **ADME studies are not strictly required to perform Phase I. However, some of the studies, i.e. bioavailability, may provide useful information at an early stage in development**
- ***In Vivo* metabolism studies should be considered where metabolism is found to be extensive and when there are strong possibilities of interspecies variation in toxicity and ADME**

Single Dose Toxicity Study

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- An evaluation of acute toxicity is normally required in two mammalian species prior to the first human exposure
- A dose escalation study is considered acceptable in dogs
- Intended clinical route of administration
- Control and three graded doses are used with 5 rats/group/sex
- Monitor conventional parameters

Repeat Dose Toxicity

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- Dose range finding studies (7-day) precede the short-term repeat dose toxicity studies
- Generally a 28-day study in two species with recovery groups
- Toxicokinetics and immunotoxicity must be included

Questions to be Answered by Preclinical Studies Preclinical Studies

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- What is the relationship of the dose to the
- biologic activity?
- What is the relationship of the dose to the
- toxicity?
- Does the route and/or schedule affect
- activity/toxicity?
- What risks can be identified for the clinical
- trial?

Reproductive Toxicology
Biologics

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- Studies defined in the ICH S5 and M3 documents are not the standard default
- Embryo-fetal toxicity studies are conducted for most compounds
- Need for fertility and pre-and post-natal development influenced by product, patient population and indication
- Timing of studies to clinical trials is flexible
- Limitations due to animal models (relevant species and immunogenicity)

Carcinogenicity
Biologics vs. Small Molecules

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- Chronic administration (2 years) to rodents and assessment of tumor formation
- Small Molecules
  - Performed for all products that are expected to be administered continually for 6 months to humans
  - 2 studies, mouse and rat
  - Due upon submission of NDA
- Biologics
  - Typical carcinogenicity studies not usually performed for biologics, unless scientifically justified
  - Limitations: animal models and immunogenicity
  - Biologics may increase cellular proliferation and promote tumor growth
  - *In vitro* and *in vivo* studies to assess growth of tumor and normal cells

Where Do I Go for Help?

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- International
  - International Conference on Harmonisation(ICH) Documents
  - http://www.ich.org/cache/compo/276-254-1.html
- CDER/CBER specific
- Internally generated documents submitted for public comment

- http://www.fda.gov/cder/guidance/index.htm
- http://www.fda.gov/cber/guidelines.htm

ICH Documents: Relevant to Biologics

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- ICH S6: Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals
- ICH S5A &B: Detection of Toxicity to Reproduction for Medicinal Products
- ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals
- ICH M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials
- ICH Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process

Take Home Points

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- Biologics and small molecules are inherently different
  - Microheterogeneity/ comparability
  - Relevant species
  - Immunogenicity
  - Clearance
- These differences are reflected in the nonclinical developmental strategies
- “One size fits all approach” not valid
- Guidances for nonclinical development can be found on CDER and CBER websites