Dose finding and clinical development of medicines
Lyon 2011

Sándor Kerpel-Fronius, M.D., D.Sc.
Semmelweis University
Department of Pharmacology and Pharmacotherapy
Budapest, Hungary
Email: kerfro@pharma.sote.hu
Items to be discussed

- Relevance of non-clinical data to the clinical development plan and risk extrapolation
- Early clinical development plan
  - The concept and use of biomarkers in drug development
  - Exploratory phase 0 trials
  - Phase I and II human trials
- The significance of Proof of Concept (POC) in the development of new medicines
- Impact of results on planned therapeutic application
Revolution in drug development and application

Cooperative effort

Basic science
Clinical research
Regulation
Health care

Diagnostic technology

Medical devices and drug combinations

Medical technology

Prevention methodology

Drugs

Pharmaceutical treatment

Kerpel-Fronius S.:
The process of drug development

Preclinical Studies
Pharmacology
- In vitro
- In vivo
Toxicology
Pharmacokinetics

Toxicokinetic studies
ADME
Drug concentration

Clinical Studies
Human tolerance
Clinical efficacy
Proof of clinical efficacy
Pharmacokinetics

Kerpel-Fronius S.
Phases of human drug development

**Phase I**
- Human tolerance (HT)
- No. of subjects: < 100

**Phase II**
- Pharmacokinetics (PK)
- <500

**Phase III**
- Quality of life and pharmacoeconomic studies
- <5000

**Phase IV**
- Marketing authorization
- >10000

**Postmarket controlling studies**
- Observational studies
- Prospective, randomized, comparative trials

**No. of subjects**
- Phase I: < 100
- Phase II: < 500
- Phase III: < 5000
- Phase IV: > 10000
Phases of human clinical drug development

Phase I human tolerance and pharmacokinetic studies

- Titration of the clinically applicable dose range
- Description of the human pharmacokinetics and metabolism
- Number of subjects is small (20-100)
- Usually healthy volunteers, in special cases patients (e.g., oncology)
Phases of human clinical drug development

Phase II study

- Description of the clinical efficacy of the drug
- Titration of the clinically effective dose range
- Few, selected patients with typical symptoms and without interfering conditions not connected to the disease
- Number of subjects is small (50 - 200)
The application of allometric principle in the extrapolation of drug doses from animals to humans

- Allometry is the science studying the differential growth rates of the parts or process of a living organism
- Allometry also refers to change in proportion of body size (mass and volume) and physiological processes relative to body area
<table>
<thead>
<tr>
<th>Species</th>
<th>$k_m$ value mg/kg to mg/m$^2$</th>
<th>Multiplication Factor (animal to human) mg/kg to mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Child (20 kg)</td>
<td>37 25</td>
<td>--</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>0.08</td>
</tr>
<tr>
<td>Hamster</td>
<td>5</td>
<td>0.13</td>
</tr>
<tr>
<td>Rat</td>
<td>6</td>
<td>0.16</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>8</td>
<td>0.22</td>
</tr>
<tr>
<td>Rabbit</td>
<td>12</td>
<td>0.32</td>
</tr>
<tr>
<td>Dog</td>
<td>20</td>
<td>0.54</td>
</tr>
<tr>
<td>Monkeys Baboon</td>
<td>12 20</td>
<td>0.32 0.54</td>
</tr>
<tr>
<td>Micro-pig</td>
<td>27</td>
<td>0.73</td>
</tr>
<tr>
<td>Mini-pig</td>
<td>35</td>
<td>0.95</td>
</tr>
</tbody>
</table>

**Conversion of animal to human equivalent dose (HED)**


Examples of calculation:
1. Mouse dose to human dose using $k_m$ value:
   Mouse dose: 1mg/kg
   1mg/kg x 3 = 3mg/m$^2$
   3 mg/m$^2$ :37 = 0.08mg/kg

2. The same using multiplication factor
   1mg/kg x 0.08 = 0.08mg/kg
Flow chart for selecting MRSD


**Determination of NOAEL mg/kg in toxicity studies**

- **Is there a justification to Extrapolate NOAEL to HED?**
  - **YES**
    - HED (mg/kg) = NOAEL (mg/kg)
    - Or other appropriate normalization
  - **NO**
    - Convert animal NOAEL to HED based on body surface
    - Select NOAEL from the most appropriate (sensitive) species
    - Choose safety factor (SF) and divide HED by this factor (usually = 10)

**Maximum recommended starting dose (MRSD)**

Kerpel-Fronius S.
No adverse event level (NOAEL): the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group. In this context, adverse effects that are biologically significant (even if they are not statistically significant) should be considered in the determination of the NOAEL.

The definition of the NOAEL, in contrast to that of the NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern.
Other dose levels determined in toxicologic studies

- The functional difference of NOAEL and LOAEL as compared to the other levels determined in toxicological studies, NOEL, LOEL, MTD
  - No observed effect level (NOEL) refers to any effect, not just an adverse one, although in some cases the two might be identical
  - Lowest observed adverse effect level (LOEL) and maximum tolerated dose (MTD). Both concepts are based on findings of adverse effects in general
No adverse effect level (NOAEL) used for determining MRSD


- The findings useful for determining NOAEL
  - overt toxicity (e.g., clinical signs, macro- and microscopic lesions)
  - surrogate markers of toxicity (e.g., serum liver enzyme levels)
  - exaggerated pharmacodynamic effects

- An adverse effects in animals used to define a NOAEL for the purpose of dose-setting should be based on an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase I clinical trial conducted in adult healthy volunteers.
No adverse effect level (NOAEL) used for determining MRSD


- Increasing the safety factor might be necessary if
  - Steep dose-response curve
  - Severe and/or irreversible toxicity
  - Unexplained mortality in toxicity studies
  - Toxicities occurring without warning symptoms
  - Variable, poor bioavailability of the drug in the different species
  - Nonlinear pharmacokinetics
  - Novel therapeutic targets
Flow chart for selecting MRSD


Determination of NOAEL mg/kg in toxicitiy studies

Is there a justification to Extrapolate NOAEL to HED?

Is there a justification to extrapolate NOAEL to HED based on mg/kg

YES

HED (mg/kg) = NOAEL (mg/kg)
Or other appropriate normalization

Convert animal NOAEL to HED based on body surface

Select NOAEL from the most appropriate (sensitive) species

Choose safety factor and divide HED by this factor (usually = 10)

Maximum recommended starting dose (MRSD)
No adverse effect level (NOAEL) used for determining MRSD


- Scaling between species based on mg/m^2 is not recommended for
  - Drugs administered by topical, intranasal, subcutaneous, intramuscular, etc routes for which the dose is limited by local toxicities. Such therapeutics should be normalized to concentration or amount of drug at the application site.
  - Therapeutics administered into anatomical compartments that have little subsequent distribution outside of the compartment
  - Proteins administered intravascularly with Mr > 100,000 daltons. Such therapeutics should be normalized to mg/kg
  - Exposure parameters which highly correlate across species on a mg/kg basis
Phase I protocol design. Selection of the study population

- Patients or healthy volunteers?
  - The risk, especially immediate and long term toxicity inherent in the type of the medicinal product.
  - The expected width of the therapeutic window
  - The relative presence of the target in healthy volunteers and in specific disease groups (e.g. cancer patients)
  - Potential pharmacogenomic difference between the targeted patient group and the healthy volunteers
  - The higher ability of the healthy volunteers to tolerate side effects
Safety of the phase I trial

- Specific plan for the monitoring of various expected side effects should be developed in advance
- A treatment strategy of the expected side effects should be described in the protocol
- Stopping rules for the termination of dose escalation should be defined
- Appropriate clinical facilities with resuscitation equipment and trained personal for treating serious side effects are mandatory for performing the trial
- Arrangement with an easily accessible intensive care unit should be made
Various dose escalation arrangements

Economic but relatively more dangerous dose escalation scheme

First dose level

Next dose level

Next dose level

Next dose level

Less economic but safer dose escalation scheme

Pilot patient at first or higher dose levels

Next dose level

Next dose level

Next dose level

Next dose level
Dose escalation schemes

Renczes G. In Rencszes G, Lakner G and Antal J. Handbook of Clinical Trials
SringMed, 2009
High risk medicinal agents

Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products
EMEA/CHMP/SWP/294648/2007
Antigen presentation linked to Major Histocompatibility Complex (MHC)
Increased expression of B7 costimulator receptor (CD80/86) on APC which binds to the constitutively expressed T-cell CD28 receptor

*T cell expansion*

Following T cell activation the expression of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is induced on T-cells. This CD28 homologue binds to B7 costimulator receptor and terminates T cell activation

*Inhibition of T cell expansion*
Co-stimulation in adaptative immune response

- Step 1: T-cell activation
  - Presentation of antigen linked to major histocompatibility complex (MHC) on the surface of the antigen presenting cells (APC)
  - Recognition of the antigen by T-cells and its binding to the CD 28, T-cell receptor (TCR)

- Step 2: Co-stimulation, needed to the full activation of T-cells
  - The best understood mechanism is the binding of the inducible B7 (CD 80/86) co-stimulator on the surface of the APC cells to the constitutively expressed T-cell (CD28) receptor
  - Proliferation of T-cells (stimulated further by increased IL-2 level)

- Step 3: Termination of T-cell proliferation
  - Induction of cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) on the T-cells
  - This CD28 homologue binds more avidly to the B7 costimulator receptor of the APC, hereby displaces T-cell (CD28) receptor
  - The costimulation and T cell proliferation is terminated
The TGN1412 phase I trial tragedy

- **TGN1412** (CD28-SuperMAB®) humanized monoclonal antibody exerting immunomodulatory activity
- Superagonist to the CD28 receptor of the T cells which fully activates T cells without the binding of an antigen
- Increased activation of regulatory T cells leading to immunosuppression
- Pronounced T cell activation leads to cytokine storm in animals
- 50 mg/kg, the maximum dose used in primates was considered to be the NOAEL
- Starting dose was 1/500 of the NOAEL, 0.1 mg/kg
- This dose will, however, bind all CD28 receptor available in the body as might be proven by antibody titration experiment
- Result, long, possible life-long immunosuppression with increased risk for developing malignancy
TGN1412. The dynamics of the events
Suntharalingam G et al. NEJM 355:1018, 2006
TGN-1412 laboratory results

Suntharalingam G et al. NEJM 355:1018, 2006

Graphs showing the levels of various cytokines (TNF-α, Interferon-γ, Interleukin-10, Interleukin-8, Interleukin-6, Interleukin-4, Interleukin-2, Interleukin-1β, Interleukin-12p70) over days after infusion.
TGN-1412 Laboratory results
Suntharalingam G et al. NEJM 355:1018, 2006
TGN-1412 Laboratory results
Suntharalingam G et al. NEJM 355:1018, 2006
Expert Scientific Group on Phase one clinical trials
Final report „Duff report”

30th November 2006

www.tsoshop.co.uk
Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products
EMEA/CHMP/SWP/294648/2007

- The guideline applies to all new chemical and biological investigational medicinal products except gene and cell therapy medicinal products.
- It covers non-clinical issues for consideration prior to the first administration in humans and the design and conduct of trials in the initial phase of single and ascending doses during the clinical development.
- Concerns for high risk may be derived from particular knowledge or lack thereof regarding
  - The mode of action
  - The nature of the target
  - The relevance of animal models
Risk factors: mode of action

- Target which is connected to multiple signaling pathways (pleiotropic effect) leading to multiple pharmacologic effects
- Targets which are ubiquitously expressed in the body
- Pharmacologic effect leading to biological cascade or cytokine release not sufficiently controlled by physiological feed-back mechanisms ((e.g. a super-agonist to CD28)
- Novelty of the substance, no previous human experience with similar structures
- Serious toxicity produced by the substance in genetically modified animals (Transgenic, knock-out animals, etc)
Risk factors: nature of the target and the relevance of animal models

- **Limited knowledge on the target**
  - The structure, tissue distribution, extent of expression
  - Cell and/or disease specificity
  - Biological effect, down-stream signaling

- **Relevance of animal species and models**
  - Qualitative and quantitative differences between the target expression and distribution
  - Differences of the biological effects, in the linked signaling pathways in different species
  - High species specificity of the biological substance. The use of transgeneic animals expressing the human target might be necessary
  - Similar response of animal and human cell lines *in vitro* does not guarantee similar *in vivo* response
  - Human specific proteins might induce Ab formation, interfering with the evaluation in animals
Determination of the human starting dose

- A weight-of-evidence approach should involve integration of information from all in vivo, ex vivo and in vitro studies into the decision-making process (Concentration-response curves, target binding and receptor occupancy, etc).

- In general the No Observed Adverse Event Level (NOAEL) adjusted with allometric factors and reduced/adjusted by appropriate safety factor.

- With substances with identified high risk factors Minimal Anticipated Biological Effect Level (MABEL) is recommended which is the anticipated dose level leading to a minimal biological effect level in humans. MABEL might be reduced/adjusted by appropriate safety factor.

- When using this approach, potential differences of sensitivity for the mode of action of the investigational medicinal product between humans and animals, need to be taken into consideration e.g. derived from in-vitro studies.
Minimum Anticipated Biological Effect (MABEL)

Figure of P.Loyd Novartis, Basel
Flow chart for selecting MRSD in case of high risk medicinal agents


Determination of MABEL mg/kg in toxicity studies

Is there a justification to Extrapolate MABEL to HED?

Is there a justification to extrapolate MABEL to HED based on mg/kg

YES

HED (mg/kg) = MABEL (mg/kg)
Or other appropriate normalization

Convert animal MABEL to HED based on body surface

Select MABEL from the most appropriate (sensitive) species

Choose safety factor and divide HED by this factor (usually = 10)

Maximum recommended starting dose (MRSD)
Precautions to apply between doses and within the cohort

- The first, single dose should be received by a single subject.
- Further dose administration should be sequential within the cohort to mitigate the risk.
- Criteria should be pre-specified in the protocol that will be used to identify and mitigate the risk of progressing to a subsequent cohort at higher dose level.
- Administration in the next cohort should not occur before all the participants in the previous cohort have been treated and data/results from those participants were reviewed.
- Unanticipated pharmacological responses may require a revised dose escalation.
- Time intervals between cohorts should be guided by non-clinical and clinical PK and PD data.
Dose escalation schemes

- **Geometric series**
  \[1, 2, 4, 8, 16, \ldots\]

- **Fibonacci series**
  \[1, 2, 3, 5, 8, 13, \ldots\]

- **Modified Fibonacci series**
  \[1, 2, 3, 5, 7, 9, \ldots\]

- **Linear**
  \[1, 2, 3, 4, 5, 6, \ldots\]
Various dose escalation arrangements

Economic but relatively more dangerous dose escalation scheme

- First dose level
- Next dose level
- Next dose level
- Next dose level

Less economic but safer dose escalation scheme

- Pilot patient at first or higher dose levels
- Next dose level
- Next dose level
- Next dose level
- Next dose level
Drug development in the 21st century
Modern drug development and application

Cooperative effort

Basic science

Clinical research

Regulation

Health care

Diagnostic technology

Medical devices and drug combinations

Drugs

Prevention methodology

Medical technology

Pharmaceutical treatment

Kerpel-Fronius S.
Target Product Profile (FDA)

- Definition of the product candidate (What will be developed?)
- Route of administration
- Indication
- Specificity
- Target population
- Dosage and schedule of administration
- Key efficacy claims
- Key safety claims
Interactions between patients, medical investigators, pharmaceutical industry

Trial subject
Medical investigator

Regulatory Agency

Pharmaceutical industry
Significant emerging new technologies
Source: Strategic Planning Pharma

- Protein chips
- Transgenic animals
- Bio-informatics
- Chemo-informatics
- In silico experimentation
- Functional genomics
- Molecular modelling
- Pharmacogenomics
- Proteomics

Source: Kerpel-Fronius S.
Major bottlenecks in pharmaceutical R&D

Biomarkers are frequently the best translational links between animal and human pathological and drug induced changes

Discovery research  Preclinical develop.  Translational medicine  Clinical develop.  Pharmaco-vigilance

Predictive pharmacology  Predictive toxicology  Patient recruitment of biomarkers  Validation of biomarkers  Benefit/Risk assessment with Regulatory Authorities

Identification of biomarkers  Efficacy  Safety
Translational medicine

- Translational medicine is the integrated application of
  - innovative pharmacology tools
  - biomarkers
  - clinical methods
  - clinical technologies
  - study designs

- Goal
  - improve disease understanding and confidence in human drug targets
  - increase confidence in drug candidates,
  - understand the therapeutic index in humans,
  - enhance cost-effective decision making in exploratory development and increase phase II clinical trial success
**FDA: Biomarker definition**

- A characteristic that is objectively measured and evaluated as an indicator of
  - normal biologic processes
  - pathogenic processes, or
  - pharmacologic responses to a therapeutic intervention

- **Biomarker qualification** is a process to prove that the analytical data obtained are indicative and predictive for the conditions or endpoints selected
Biomarkers are measurable characteristics that reflect physiological, pharmacological, or disease processes.

**Biomarkers: A simple conceptual architecture**

*Courtesy of Bühler F, EFCPM, Basel,*

**Pharmacodiagnostic Biomarkers**
- Treatment eligibility
- Response prediction

**Pharmacological Biomarkers**
- Pharmacodynamic markers
- Pharmacokinetic markers
- Mechanism of action markers

**Disease Biomarkers**
- Predisposition
- Early detection
- Staging, prognosis,
- Monitoring/Recurrence

Biomarkers are measurable characteristics that reflect physiological, pharmacological, or disease processes.
Clinical endpoints

- **Clinical endpoints** are characteristics or variables that reflects the condition of the patients, how they feel, functions or survives.

- **Clinical validation of biomarkers** is a highly formalised process intended to prove that the assay provides reliable data and is suitable for the intended purpose in the clinical practice.
  - Biomarkers can be used as surrogate endpoints following appropriate validation.

- **Surrogate endpoints** are intermediate outcome(s) having a causal relationship with the pathological condition or outcome caused by the disease, the changes of which give information about whether the natural course of the disease is affected by the applied treatment.
Evaluation of the outcome of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy of treatment</td>
</tr>
<tr>
<td>Side effects of treatment</td>
</tr>
<tr>
<td>Psychologic, social effects of treatment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measuring methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical parameters</td>
</tr>
<tr>
<td>Intensity scale of side effects</td>
</tr>
<tr>
<td>Quality of life questionnaire</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Components of the outcome of the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
</tr>
<tr>
<td>Death</td>
</tr>
<tr>
<td>Functional disability</td>
</tr>
<tr>
<td>Discomfort</td>
</tr>
<tr>
<td>Damage caused by treatment</td>
</tr>
<tr>
<td>Economic situation</td>
</tr>
</tbody>
</table>
Biomarkers of Nephrotoxicity (Example from ILSI-HESI)

GLOM - collagen
IV

PCT - αGST

PCT - clusterin

http://www.irvingcrowley.com/cls/nephron.htm

DCT - μGST

LOH - RPA-2

CD - RPA-1

Courtesy of Kirstin Meyer
EMEA/FDA evaluation of urinary biomarkers for sensitive detection of kidney toxicity

Nephrotoxicity biomarkers experience EMEA/FDA: Doc Ref. EMEA/679719/2008 Rev.1

- **Non-clinical studies**: Urinary kidney injury molecule (KIM-1), albumin, total protein, clusterin, beta2-microglobuline, urinary Cystatin C protein, Trefoil factor 3 protein (TFF3) are all capable for detecting acute drug-induced glomerular or tubular nephrotoxicity in non-clinical studies.

- They provide additional and complementary information to BUN and serum creatinine but further data are needed for detecting reversibility.

- **Human studies**: for detecting human drug induced nephrotoxicity and the demonstration of its reversibility further clinical evaluation is needed.

- They application for clinical trials is not yet varanted.
Relative Operating Characteristic (ROC) curve of BMs for pathology of kidney tubular damage

Nephrotoxicity biomarkers experience EMEA/FDA: Doc Ref. EMEA/679719/2008 Rev.1

Perfect classification

Tubular Degeneration / Necrosis / Apoptosis / Cell Sloughing

S1 - S3 + non-localized

True positive

Random guess line

Better

Worse

False positive

Area Under Curve:
Random = 0.5
Creatinine = 0.72
BUN = 0.75
Kim-1 = 0.89
Clusterin = 0.85

Doc. Ref. EMEA/679719/2008 Rev. 1

135 Diseased
604 (595) Controls

Kerpel-Fronius S.
Relative Operating Characteristic (ROC) curve of BMs for pathology of kidney glomerular damage

Nephrotoxicity biomarkers experience EMEA/FDA: Doc Ref. EMEA/679719/2008 Rev.1

- Area Under Curve:
  - Random = 0.5
  - Creatinine = 0.55
  - BUN = 0.74
  - Tot. Protein = 0.86
  - B2-Microglob. = 0.89
  - Cystatin C = 0.90

- 41 Diseased
- 698 Controls
Learn-Confirm Paradigm
Sheiner L.B. Framework for Optimal Drug Development

**Learning**
- mechanistic (causal) understanding of product-exposure-response relationships

**Confirming**
- demonstrating evidence of mechanism, therapeutic concept, safety & effectiveness

Lewis B. Sheiner, M.D., Ph.D.
Physician and Scientis
1940 – 2004
The fate of active ingredients in the body

Changing parameter
- Amount liberated
- In vitro dissolution %
- In vivo ????
- Absorbed amount

Concentration in the body

Process
- Liberation
- Absorption
- Distribution
- Metabolism
- Elimination

Research field
- Biopharmaceutics
- Pharmacodynamics
- Pharmacokinetics

Courtesy of I. Antal, Budapest

Biological response

Kerpel-Fronius S.
The concept of Phase 0 clinical trials
Position paper on non-clinical safety studies to support clinical trials with a single microdose. CPMP/SWP/2599/02, 2004

**Phase 0**
- Extended single dose toxicology (14 days)
- 1/1000 toxic dose level
- Very small human dose
- < 100 µg, 1/100 of expected pharmacologic dose
- Short duration, few patients

**Goal:** Verification of the mechanism of action using biomarkers: enzyme inhibition, receptor binding, imaging; PK and PD studies

Bridge between non-clinical drug discovery and clinical development
Drug discovery
Leading molecule selection
Target identification
Pharmacologic, pharmaceutical optimization

Learning phase

Phase 0
Phase I
Phase II
Phase III

Confirmation phase

Feedback
Optimization
Materials of human origin

Translational medicine biomarkers

Proof of concept

Marketing authorization

Kerpel-Fronius S.
Proof of Concept (POC)

- To test both the compounds and the identified mechanisms or targets in actual patients before full development
Proof of concept (POC)
Cartwright ME et al. Clinical Pharmacology and Therapeutics, 87:278-85, 2010

- POC is the earliest time point during drug development when based on the weight of evidences it might be concluded with great probability that

- *the most important conditions for success are present*

- *the most important determinants of failure are absent*
Effectiveness

- The mechanism of action was proven and the successful effect on the outcome of the disease was demonstrated.
- The target population was determined and the linked diagnostics had been worked out.

Pharmacokinetics

- The expected pharmacodynamic actions could be demonstrated at achievable and tolerable drug plasma level.
- The drug dose and the schedule of administration had been defined.

Safety

- The positive and negative effects exerted on other sites of action are of acceptable intensity.
- The clinical effects are in line with the non-clinical observations (translational medicine).
Proof of concept (POC)
Cartwright ME et al. Clinical Pharmacology and Therapeutics, 87:278-85, 2010

- **Biopharmacy**
  - Suitable drug formulation and industrial scale production method had been developed
  - The production costs are acceptable

- **Marketing**
  - The characteristics of the product can be adequately distinguished from the competitors
  - Acceptable price and reimbursement can be anticipated

- **Marketing authorisation**
  - Approvable indication
  - The target population and the selected end points of the patient population are relevant and acceptable
Translational medicine in drug development

Critical path

Basic research
- Drug prototype discovery and design

Non-clinical development

Clinical development
- Phase 1
- Phase 2
- Phase 3

Marketing Authorization

Identification of disease target
- Optimization of molecular targeting

Safety optimization
- Proof of concept
- Broad confirming clinical trials

Kerpel-Fronius S.: 63
Modern concept of drug development

Predictive non-clinical research
- PK/PD parameters
- Pathway analysis
- Dose selection
- Biomarker identification
- Analogue development

Marketing
- Authorization
- Indication
- Side effect profile
- Companion biomarkers, diagnostics

Clinical research

Proof of concept in humans
- Mechanism of action
- Confidence in safety
- Biomarker validation
- Multiple effects in humans?

Confirmation
- Use of biomarkers for patient selection, stratification
- Efficacy/Safety
- Benefit/Risk

Kerpel-Fronius S.
FDA: Phase 0, exploratory IND for anticancer agents

In US: 7 days long repeated administration is possible
FDA: Phase 0, exploratory IND for anticancer agents

ABT-888 a DNA repair inhibitor. The expected active plasma level 210 nmol/l. Enzyme activity was measured in Peripheral Blood Mononuclear Cells (PBMC) and tumor.
Emerging ethical issues
The harmonization of contradictory aims, needs and ethical principles

**Freedom of research**

- Safety and the protection of personal rights and dignity
- The value of the scientific results for the society

**Freedom of research**

The value of the scientific results for the society

Safety and the protection of personal rights and dignity

**Freedom of research**
The harmonization of ethical requirements associated with the collection of human biological material for genetic research within clinical trials

Ethical guidelines for Clinical research

Ethical guidelines for Genetic research

„Combined research”
Clinical drug trial + Genetic research

How to handle ethical issues satisfying both types of research?
Identifiability of biological materials of human origin

Steering Committee on Bioethics (CDBI) Recommendation of the Committee of Ministers to member states on biological materials of human origin.

- **Identifiable biological materials**: the person concerned can be identified alone or in combination with associated data
  - In the case of **coded material** the investigator has direct access to the code
  - Linked anonymised (pseudoanonymised) **material**: the user has no access to the code

- **Non identifiable (unlinked anonymised) biological material**: the identification of the person is made permanently impossible
Information and consent for the primary and secondary use of biological material and related personal data

- The information should be specific and as detailed as possible with regard to any foreseen research uses and the choices available in this respect.

- The consent should specify the options:
  - A general consent is recommended when the future uses cannot be clearly defined.
  - Possibility of refusing or future opting out.
  - Consent limited to only unlinked anonymised use, or limited only to specific research projects.
How to satisfy the ethical requirements of clinical and genetic research jointly?

- Do we adequately address the ethical issues concerning biological material of human origin in „combined research trials”?
  - Do we need separate committees to provide opinions related to the clinical and genetic research aspects of a combined protocol?
  - Do we need separate informed consents for the clinical trial and genetic research?
  - Do we need a special European guidance for „combined research trials”?
Futuristic concept of R&D
PriceWaterhouseCoopers
Pharma 2020: The vision
Molecular development:
• Translational medicine
• Molecular diagnostics
• Biomarker based targeted therapy
Clinical development:
• Limited launch with live licence

Continuous stimulation of the development by feedback loops
Biological Medicinal Products
First biosimilar product authorized by EMEA

Aspirin
Mw: 180

Human growth hormone
(Somatropin, Genotropin®)
MW: 22 000 dalton, 197 amino acids
4 helices which interact with GHR
Biosimilar product: Omnitrope®
Grouping of medicinal products
(Biological medicinal products)

❖ Chemical medicinal products
  ➢ Medicines containing chemical active ingredient(s)
  ➢ Small molecules which can be accurately characterized chemically

❖ Biological medicinal products
  ➢ Medicines containing biological material, macromolecules produced by or extracted from a biological source
  ➢ Cannot be fully characterized due to the structural complexity of the macromolecules
  ➢ There is a need to use a combination of physico-chemical-biological testing, together with process control, to define their quality and characteristics
A cell as production facility

The cellular factory produces the required protein, but *the precise control of the production is uncertain*

The product
A cell specific mixture of protein molecules with different length and glucosylated variously
Erythropoetin szerkezete
## Function of glycoprotein glycans

Hermeling et al., Pharmaceutical Res. 21:897-903

<table>
<thead>
<tr>
<th>Physico-chemical</th>
<th>Modify solubility, electric charge, mass size, viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control protein folding, stabilization of conformation</td>
</tr>
<tr>
<td></td>
<td>Confer thermal stability and protects against proteolysis</td>
</tr>
<tr>
<td>Biological</td>
<td>Regulate intracellular trafficking and localization</td>
</tr>
<tr>
<td></td>
<td>Determine pharmacokinetic properties ($t_{1/2}$)</td>
</tr>
<tr>
<td></td>
<td>Modulate activity</td>
</tr>
<tr>
<td></td>
<td>Act as cell surface receptor for different ligands</td>
</tr>
<tr>
<td></td>
<td>Participate in cell-cell interaction</td>
</tr>
</tbody>
</table>
Cross reactivity between mAbs against differently glycosylated forms of rhGM-CSF


- Abs against rhGM-CSF produced in yeasts were observed in 4/13 patients
- These Abs recognized rhGM-CSF produced in bacteria
- Recognition occurred in CHO cells only after O-cleavage of the sugar moiety
- O-glycosylation protects the amino acid backbone in the natural and in the product derived from CHO cells
At all stages of the production, purification and pharmaceutical formulation, as well as during storage and preparation for administration, the complex spatial structure, the glycosylation pattern of the protein products can undergo changes resulting in altered biological activity and immunogenicity.

Clinical consequences of immunogenicity

Biological medicine (protein) → Antibody production

- Neutralizing Abs
- Decreased clinical efficacy

Neutralization of native proteins
Impairment of physiological functions

Pharmacokinetic differences
Bioequivalence studies might be interfered with

No effect
Documentation needed for marketing application

The requirements for generic and biosimilar agents are similar.
Similar biological medicinal products
Clinical advantages and problems

**Proven identical and/or similar properties:**
- Identical amino acid composition
- Similar pharmacokinetic properties
- Similar pharmacologic effects

**Dissimilar properties:**
- Post-translational modifications
- Immunogenic properties
- The clinical significance of the differences are uncertain

Biosimilar medicines
The significance of bioequivalence in biosimilar substitution

Equal plasma level → Similar active substance → Similar clinical effect

- Equal plasma levels of biosimilar drugs lead to similar but not to equal clinical effects

\[
\log\text{AUC}_1 / \log\text{AUC}_2 = 1 \quad (0.80 - 1.25)
\]
The scheme of comparative pharmacokinetic and pharmacodynamic evaluation of filgrastim
Filgrastim Ratiopharm. EPAR. EMEA/H/C/824

- 5 and 10 μg/kg, i.v. and s.c. administrations, wash-out period 2 weeks
- 56 healthy male volunteers
### Comparative Pharmacokinetic and Pharmacodynamic Studies in Healthy Volunteers

**Filgrastim Ratiopharm. EPAR. EMEA/H/C/824**

#### 5 µg/kg, s.c.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Concentration (ng/ml)</th>
<th>ANC (10^9/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PK study**

**PD study**

**Figure:**

- **Comparison of Concentration (ng/ml) and ANC (10^9/l) over Time (h).**
- **PK study** at 5 µg/kg, s.c. with concentration and ANC over time.
- **PD study** showing ANC response to treatment T and R over time.

---

**Graphical Representation:**

1. **Concentration (ng/ml) vs. Time (h):**
   - Data points for PK study at 5 µg/kg, s.c.
   - Error bars and trend lines.

2. **ANC (10^9/l) vs. Time (h):**
   - Data points for PD study at 5 µg/kg, s.c.
   - Treatment T and R indicated.

---

**References:**

- KERPHEL-FRONIUS S. 88
**Generic and Biosimilar medicinal products**

Guideline on similar biological medicinal products: CHMP/437/04. ff

Innovative medicinal products

- **Chemical medicinal products**
  - Chemical identity can be proven
  - Comparative bioequivalence can be used to prove safety and efficacy
  - No therapeutic comparison is needed

- **Biological medicinal products**
  - Full chemical identity at the molecular level cannot be proven
  - Comparability program using the reference drug is needed to prove similarity of quality, clinical efficacy and safety (immunogenicity!)

Follow-on medicinal products

- **Generic medicinal products**
- **Biosimilar medicinal products**
EMEA guidelines concerning the marketing authorization of biosimilar medicinal products

General guidelines

Guideline on similar biological medicinal products
CHMP/437/04, 2005

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance:
Non-clinical and clinical issues
EMEA/CHMP/BWP 42832/2005

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance:
Quality issues
EMEA/CHMP/BWP 49348/2005

Annexes are prepared for special products:
Growth hormone, insulin, erythropoietin, colony stimulating factors, etc.
EMEA guidelines concerning the marketing authorization of biosimilar medicinal products

Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins
London, 24 January 2007
Patient samples taken at appropriate time points

Negative sample

Positive sample

Surface plasmon Resonance assay (Real time interaction)

Confirmational studies for specificity

Assess correlation between measured antibody titer and biological function

Development of methods for the monitoring of clinical markers and efficacy

Binding assay

Biological assay (neutralising / blocking Abs)

The demonstration of antibodies during the development of biological medicines
Characterization of antibodies developed
Courtesy of Stanulovic V.

- Screening assay
- Confirming assay
  - Confirmation of positive assay with excess drug
- Titer determination
- Characterization assays
  - Neutralization of activity / blocking of binding
  - Isotype determination
  - Complement fixing ability
  - Epitope mapping (anti-framework, anti-CDR)
  - Determination of relative binding affinity

Screening assay

Confirmation

Titration assay

Characterization assays
Correct and incorrect clinical application of analogue és biosimilar medicines

**Incorrect**

- Starting medicine of the treatment
- Biosimilar products

**Correct**

- Any of the medicines without replacement

---

**Exchange of medicines**

- Exchange of medicines should be done only due to medical reasons and should be followed by intensive monitoring of the patients
- The therapy should be performed preferentially with the same product if there is no medical need for exchanging the drugs
Size and complexity of MAbs

Nick C. Parexel

Aspirin
180 Daltons

Insulin
5700 Daltons

MAb
150,000 Daltons
Antibodies humanized to different extents

Estimated incidence of human anti-product immunologic responses: 55 to > 80% murine mAbs; < 1 to 13% for chimeric mAbs; < 1% for fully humanized mAbs

IgG: complex and multifunctional molecule

Carter PJ. Nature Reviews Immunology, 6:343-357, 2006
Rituximab

variable, murine regions bind specifically to CD20-antigens of B-cells

human constant κ-regions

human IgG1-Fc portion
Various mechanisms of cytotoxicity

Direct Effect (e.g. apoptosis mediated by Fab part).

Target cell

Complement dependent cytotoxicity

Complement

FcR

Effector cell

Immune effector functions mediated by Fc interactions

Antibody dependent cellular cytotoxicity (ADCC)
Recommendations for the safe clinical application of biosimilar medicinal products

- It is recommended to perform a continuous treatment program of one patient with the same agent, provided the therapeutic efficacy of the drug remains satisfactory.
- The exchange of biological products should be always done by physicians and must be intensively monitored.
- The placing of biosimilar drugs into reference price groups is not recommended in order to avoid their frequent exchange due to economic considerations.
Clinical example of dose finding and the development of applicable administration schedules for cytotoxic and cytostatic agents
The “Gompertz” growth curve of tumors

- In the early phases of tumor development the growth is exponential.
- At the time of clinical diagnosis the growth is already slow, the dividing compartment is small, while the $G_0$ fraction is large.
- Surgical and/or radiological methods decrease tumor size, during regeneration many cells will enter the division cycle.
In the various tissues and tumors the sizes of the cell compartments are different.

The G_0 cells are a reservoir of cells able to divide upon stimulation.
Cell physiologic alterations required for tumor growth

- Self-sufficiency in growth signals
- Loss of sensitivity to growth-inhibitory signals
- Evasion of apoptosis
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis formation
**Principle of action of cytotoxic and cytostatic agents**

**DIVIDING CELLS**
- (growth fraction)

**CELLS ABLE TO DIVIDE** $G_0$

- **G0** Cell pool

---

**Cytotoxic treatment**
- Destroys cells in active division

**Cytostatic treatment**
- Prevents cells from entering into active division
Cytotoxic and cytostatic antineoplastic agents

- **Cytotoxic antineoplastic agents** directly impair the structural, functional and/or genetic integrity of the tumor cells. According to the severity of the damage the impaired cells might live further or die.

- **Cytostatic antineoplastic agents** do not impair or kill tumor cells directly, they interfere only with cell functions required for malignant tumor growth. As a result, prolonged steady state coexistence is established between the tumor and the host organism.
## Characteristics of cytotoxic and cytostatic therapy

<table>
<thead>
<tr>
<th>Cytotoxic therapy</th>
<th>Cytostatic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causes direct cellular damage leading frequently to cell death</td>
<td>Does not cause direct cellular damage, inhibits only cell functions required for malignant growth</td>
</tr>
<tr>
<td>Tumor shrinkage is frequently observed if cell death is dominating</td>
<td>Only tumor growth is inhibited, tumor shrinkage is only sometimes observed</td>
</tr>
<tr>
<td>Has frequently poorly defined targets</td>
<td>The molecular targets are well characterized</td>
</tr>
<tr>
<td>Host and tumor cells are equally damaged, toxicity is frequently severe</td>
<td>Host toxicity compared to antitumor effect is usually mild</td>
</tr>
</tbody>
</table>
# Characteristics of cytotoxic and cytostatic therapy

<table>
<thead>
<tr>
<th>Cytotoxic therapy</th>
<th>Cytostatic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent therapy can be applied due to irreversible binding of the drug to the target and/or permanent cellular damage caused</td>
<td>Drug target binding is easily reversible, continuous therapy must be used</td>
</tr>
<tr>
<td>Continuous, prolonged administration causes lethal bone marrow damage, therefore intermittent administration is preferred to allow sufficient time for host tissue regeneration</td>
<td>Continuous, prolonged therapy can be maintained due to the mild toxicity to the host. Cumulative toxicity might be more severe</td>
</tr>
</tbody>
</table>
Ligand binding induces dimerization of the intracellular kinase domain which leads to catalytic activity.

Each catalytic domain phosphorylates tyrosines on the adjacent receptor catalytic domain.

Substrates and not metabolized adapter molecules bind to the phosphotyrosines.
The effect of cytotoxic and cytostatic agents on the growth of tumors

- Continuous cytostatic treatment
- Intermittent cytotoxic treatment

- Cytostatic effect is occasionally associated with tumor regression, if tumor cells become "addicted" to the altered gene product

- Cytotoxic effect
Toxicity and antitumor effect

**A**  
*Cytotoxic treatment*  
![Diagram](image)

**B**  
*Cytostatic treatment*  
![Diagram](image)
Mechanism of action of cytotoxic agents

Block synthesis and incorporation of **purine** and **pyrimidine** nucleotides

**Antimetabolites**

**Topoisomerases I and II** relax super-coiled DNA by cutting one, respectively both strands and later religate them.

DNA

**Topoisomerase I -II inhibitors** block religation

**Cross-link formation by alkylating agents and Pt** inhibits DNA synthesis

**Microtubule**

Depolymerization to α and β tubulin

**Taxanes**

**Vinca alcaloids**

Polymerization to microtubules
Principles of cancer chemotherapy using cytotoxic agents (CT)

- **The patients should be treated with the highest possible dose safely tolerated. Lower doses will not lead to clinically meaningful tumor regression**

- **Maximal tolerated dose (MTD):** a dose causing life-threatening, rapidly reversible toxicity and/or severe, prolonged or irreversible organ toxicity

- **Clinically applicable dose:** a dose safely applicable during repeated cycles causing moderate to severe rapidly reversible toxicity and mild to moderate, prolonged or irreversible organ toxicity

- CT is usually administered in repeated cycles separated by intervals permitting the regeneration of organs whose function depends on rapid cell renewal, primarily the bone marrow and GI tract

- A treatment cycle consists of the drug administration period and the subsequent interval
The calculation of the starting human dose of cytotoxic agents

Starting dose calculation applied in the eighties
Presently calculation is made according to the principles developed for high risk substances

MELD10 = mouse equivalent LD10
LTD = low toxic dose
Dose escalation schemes

Renczes G. In Rencszes G, Lakner G and Antal J. Handbook of Clinical Trials
SringMed, 2009

- Geometric series
  \(1, 2, 4, 8, 16, \ldots\)

- Fibonacci series
  \(1, 2, 3, 5, 8, 13, \ldots\)

- Modified Fibonacci series
  \(1, 2, 3, 5, 7, 9, \ldots\)

- Linear
  \(1, 2, 3, 4, 5, 6, \ldots\)
Dose escalation of cytotoxic agents

Pharmacokinetically guided method

For safety reasons at the new dose level first only 1 patient, thereafter 2-3 pts are treated at the same dose level simultaneously.

MTD 27.7 %

20.8 33
15.6 33
11.8 33
1stTD 8.8 33
6.6 40
5.0 50
3.3 67
2.0 100
HSD 1.0 100

Modified Fibonacci scheme

Kerpel-Fronius S. 116
Cytotoxic agents kill many bone marrow (BM) cells, but the regeneration of BM is more rapid than that of the tumor.

The interval between the administration of cytotoxic agents should be longer than needed for adequate BM regeneration.
Frequently applied administration methods for cytotoxic agents

- q3w + CSF
- q3w
- q2w + CSF
- q2w
- qw
- daily
### NCI: Common toxicity criteria (CTC)


CTC Version 2.0  
Publish date: April 30, 1999

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Toxicity grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

CTC Version 2.0
Publish date: April 30, 1999
Main categories of the CTC

- Allergy/immunology
- Auditory/hearing
- Blood/bone marrow
- Cardiovascular (arrhythmia)
- Cardiovascular (general)
- Coagulation
- Constitutional symptoms
- Dermatology, skin
- Endocrine
- Gastrointestinal
- Hemorrhage
- Hepatic
- Infection/ febrile neutropenia
- Lymphatics
- Metabolic/laboratory
- Musculoskeletal
- Neurology
- Ocular/visual
- Pain
- Pulmonary
- Renal/genitourinary
- Secondary malignancy
- Sexual, reproductive function
- Syndromes (not included into previous categories)