Non-Clinical Drug Development:
With Examples from Oncology Therapeutics
Chris H. Takimoto, MD, PhD, FACP

South Texas Accelerated Research Therapeutics
San Antonio, TX

Professor (Adjunct) of Pharmacology
University of Texas Health Science Center at San Antonio
March 27, 2008
Drug Development

Drug discovery & screening
Non-clinical development
Animal scale up
Phase I studies
Phase II studies
Phase III studies

Specific examples from anticancer drug development
Overview of Anticancer Drug Development

Flow chart indicating the development of an anticancer drug from IND (chemical synthesis and formulation development to animal models for efficacy to assay development to animal PK and PD), through clinical development (Phase I, Phase II, Phase III) to NDA.
Goals of Non-Clinical Testing of Small Molecule Drugs and Biologicals

To characterize potential adverse drug effects
   Define end organ toxicities
   Define reversibility of toxicity
To characterize pharmacokinetic profile
To characterize beneficial pharmacodynamic effects
   Proof of principle
To guide safe use in human clinical studies
   To determine a safe & reasonable starting dose
   Provide monitoring guidelines for the clinical study
Provide sufficient data to conclude that patients are not exposed to unreasonable risks
Oncology drug development is changing in the new era of targeted cancer therapies
## Targeted Therapies & Preclinical Development
*(adapted from Paoletti 2005)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cytotoxic Agents</th>
<th>Targeted Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>Cell based, empirical</td>
<td>Receptor based screen, rationale</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Often unknown</td>
<td>Basis for screening</td>
</tr>
<tr>
<td>Pharmacological</td>
<td>Cytotoxic</td>
<td>Cytostatic</td>
</tr>
<tr>
<td>Effect</td>
<td>Non-selective</td>
<td>Selective</td>
</tr>
<tr>
<td>Specificity</td>
<td>Pulsed, cyclical at MTD</td>
<td>Continuous, at tolerable dose</td>
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# Targeted Therapies & Phase I Trials
*(adapted from Paoletti 2005)*

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<tr>
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<th>Cytotoxic Agents</th>
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<tbody>
<tr>
<td>Objectives</td>
<td>PK, MTD</td>
<td>Optimal biological dose (OBD), PK, PK-PD</td>
</tr>
<tr>
<td>Disease</td>
<td>All types</td>
<td>All types or target bearing</td>
</tr>
<tr>
<td>Dose</td>
<td>Toxicity-guided</td>
<td>Biomarker-guided escalation</td>
</tr>
<tr>
<td></td>
<td>escalation</td>
<td></td>
</tr>
<tr>
<td>Endpoints</td>
<td>Toxicity, MTD, PK</td>
<td>Target inhibition, OBD, PK</td>
</tr>
<tr>
<td>Design</td>
<td>Dose escalation in small cohorts</td>
<td>Dose escalation to target inhibition</td>
</tr>
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</table>
Components of Non-Clinical Drug Development

In vitro studies: Cell lines, cell-free systems (drug screening)

Drug formulation

Chemistry, Manufacturing, and Controls: Drug supply & quality

In vivo efficacy studies: Animal models and proof of principle

Non-clinical safety studies
In Vitro Study Goals: Define the Drug’s Pharmacology

Molecular mechanism of action and specific drug targets

Molecular pharmacology

Determinants of response

Intracellular pharmacodynamics

Mechanisms of drug resistance
In Vitro Study Systems

Cell-free assay for specific molecular effects
   Enzyme inhibition, receptor blockade, etc.

Yeast-based screening in genetically defined target

Mammalian cell lines: (murine, human, etc.)
Preclinical Pharmacology
In Vitro Studies of Cancer Agents (1)

Define anticancer effects
  Growth inhibition, differentiation, apoptosis, etc

Impact on defined biochemical and molecular pathways
  RNA, DNA and protein biosynthesis, signaling kinases, etc

Spectrum of antitumor activity
  Human tumor cell lines
Preclinical Pharmacology
In Vitro Studies of Cancer Agents (2)

Cellular uptake and membrane transport
   MDR, MRP, etc

Mechanisms of resistance

In vitro drug metabolism
   P450 isoenzymes

Effects on hERG channels (prolonged QT interval risk)

Preliminary protein binding studies
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Non-clinical safety studies
Drug Supply and Formulation

Drug supply: bulk chemical synthesis, natural product isolation, etc.

Good Manufacturing Practice (GMP) guidelines for pharmaceutical product manufacturing

Formulation for clinical delivery of drug: vehicles for intravenous or other routes of administration
Drug Supply Issues

Paclitaxel source from the bark and wood of the Pacific Yew tree

Early drug supply limited the amount available for initial clinical trials

Newer semisynthetic production from the needles of the Yew tree (renewable)
Drug Formulation Issues

Poor water solubility of natural products

Paclitaxel formulation in Cremophore EL™ (increased toxicity?)

Camptothecin derivatives formulated in a dimethylacetamide, polyethylene glycol and phosphoric acid vehicle

Later formulated as a lipid colloidal dispersion
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Non-clinical safety studies
In Vivo Study Goals: Animal Models

Efficacy: Proof of therapeutic principle

Toxicology: Toxicity profile

Practical Issues:
  Animal pharmacokinetics and pharmacodynamics
  Starting dose and schedule for clinical trials
Animal Models

*Proof of Principle*

Animal screening is too expensive for routine use

Efficacy in animal models of specific disease states occurs after in vitro studies

Evaluation of therapeutic index
  Toxicity versus efficacy
Ideal Animal Model

Validity
Selectivity
Predictability
Reproducibility

“There is no perfect tumor model”
Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth


Photograph of a mouse with tumor growth that was treated with endostatin. Another photograph of a mouse with tumor growth that was treated with a saline solution. The tumor on the mouse treated with saline solution appears to be much larger than the tumor on the rat that was treated with endostatin.
Animal Models in Cancer

Spontaneous tumors
   Idiopathic
   Carcinogen-induced
   Transgenic/gene knockout animals: p53, RB, etc

Transplanted tumors
   Animal tumors: Lewis lung, S180 sarcoma, etc

   Human tumor xenografts: human tumor lines implanted in immunodeficient mice (current NCI standard in vivo efficacy testing system)

   Human tumors growing in vivo in implantable hollow fibers
Human Tumor Xenografts

Athymic “nude” mice developed in 1960’s

Mutation in nu gene on chromosome 11

Phenotype: retarded growth, low fertility, no fur, immunocompromised
   Lack thymus gland, T-cell immunity

First human tumor xenograft of colon adenocarcinoma by Rygaard & Poulson, 1969
Athymic Nude Mice

Six photographs of athymic nude mice
Murine Xenograft Sites

Subcutaneous tumor (NCI method of choice) with IP drug administration

Intraperitoneal

Intracranial

Intrasplenic

Renal subcapsule

Site-specific (orthotopic) organ inoculation
Xenograft Study Endpoints

Toxicity Endpoints:
  Drug related death
  Net animal weight loss

Efficacy Endpoints
  Clonogenic assay
  Tumor growth assay (corrected for tumor doubling time)
  Treated/control survival ratio
  Tumor weight change
Xenograft Tumor Weight Change

Tumor weight change ratio (used by the NCI in xenograft evaluation)

Defined as: treated/control x 100%

Tumor weight in mg = (a \times b^2)/2

a = tumor length
b = tumor width

T/C < 40-50% is considered significant
Xenograft Advantages

Many different human tumor cell lines transplantable

Wide representation of most human solid tumors

Allows for evaluation of therapeutic index

Good correlation with drug regimens active in human lung, colon, breast, and melanoma cancers

Several decades of experience
**Xenograft Disadvantages**

Brain tumors difficult to model

Different biological behavior, metastases rare
  - Survival not an ideal endpoint: death from bulk of tumor, not invasion

Shorter doubling times than original growth in human

Less necrosis, better blood supply

Difficult to maintain animals due to infection risks

Host directed therapies (angiogenesis, immune modulation) may not be applicable
  - Human vs. murine effects
  - Ability to mimic the human tumor microenvironment is limited
Other Animal Models

Orthotopic animal models: Tumor cell implantation in target organ
  Metastatic disease models

Transgenic Animal Models
  P53 or other tumor suppressor gene knockout animals
    Endogenous tumor cell development
    May be of high value for mAb therapies

Low passage xenograft tumors
  Direct implantation from patients to animals
Non-Clinical Efficacy Testing
The FDA Perspective
(J. Leighton, FDA ODAC Meeting, March 13, 2006)

Pharmacological activity assessed by models of disease are generally of low relevance to safety (IND) and efficacy (NDA) decisions

- Efficacy in vivo and in vitro from non-clinical studies may not dependably predict clinical efficacy
  - Heterogeneity of disease
  - Interspecies differences in ADME
  - Role of immune system

Pharmacology studies are useful for:

- Assessing an appropriate schedule (daily, weekly, q3wks)
- Justification for a drug combination
- Understanding effect at a molecular target
  - Examine receptor specificity
  - Identifying and evaluating biomarkers
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Non-clinical safety studies
Non-Clinical Safety Studies

Safety pharmacology
Pharmacokinetic and toxicokinetics studies
Genotoxicity studies
Reproductive toxicity studies
Carcinogenicity studies

**Formal toxicology studies**
- Single dose toxicity studies
- Repeated dose toxicity studies

Excellent reference:
Non-Clinical Toxicology Studies

GLP Toxicology is expected
   Use the clinical schedule, route, and formulation

Single dose acute toxicity studies required in 2 mammalian species prior to FIH studies
   Classically rat and dog for small molecules
   Non-human primates for biologicals

Repeat dose toxicity required for anticipated duration of clinical use for most non-oncology agents
   3 mo. toxicity for ≤ 3 mo. clinical study

Recommendations for anticancer agents may differ from other therapeutic areas
Expected Toxicology Testing for Phase I Oncology Drug Studies  
(J. Leighton, FDA ODAC Meeting, March 13, 2006)

<table>
<thead>
<tr>
<th>Clinical Schedule</th>
<th>Preclinical study schedule *</th>
</tr>
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<tbody>
<tr>
<td>Every 21 d</td>
<td>Single dose study</td>
</tr>
<tr>
<td>Every 14 d</td>
<td>2 doses, 14 d apart</td>
</tr>
<tr>
<td>Weekly x 3, week off</td>
<td>Weekly x 3</td>
</tr>
<tr>
<td>Daily x 5, break</td>
<td>Daily x 5</td>
</tr>
<tr>
<td>Continuous daily</td>
<td>Daily for 28 days</td>
</tr>
</tbody>
</table>

* Study schedule does not include a recovery period

28 day toxicology is generally sufficient for DRUG trials extending beyond 28 days
Non-Clinical Toxicology Studies For Oncology Drug Combinations

May not be necessary for testing in advanced cancer patients

May exclude if:
   No PK, PD, or metabolic interactions anticipated

   Drugs are not packaged as a combination

   All components well studied individually
**Single Dose Toxicity Studies**

Dose escalation study may be an alternative to a single dose design

Dose range should include maximally tolerated dose (MTD) and no adverse effect level (NOAEL)

**Standard design**

Early sacrifice at 24 to 48 hr and after 14 days
Repeated Dose Toxicity Studies

Duration of repeated dose studies related to duration of anticipated clinical use

Use same schedule and duration

Typically 14-28 days

Should include recovery group

Use can support repeat dose clinical studies
Non-Clinical Toxicology Endpoints

Ongoing Endpoints
   Clinical signs, behavior
   Body weights and food consumption
   Clinical pathology (in larger species)
      Hematology
      Chemistry panels
   Toxicokinetics

End of Study Endpoints
   Macroscopic changes at necropsy
   Organ weights
   Histopathology of all organs
Maximum Recommended Starting Dose (MRSD) for FIH Trials

Determination of the No Observed Adverse Effect Level (NOAEL)

Conversion of NOAEL to Human Equivalent Dose (HED)

Selection of the most appropriate animal species

Application of a safety factor to determine MRSD

Compare MRSD with pharmacologically active dose (PAD)

_FDA Guidance for Industry July 2005_
Selection of MRSD
(FDA Guidance 2005)

Flow chart showing the following steps in the selection of MRSD.

1. Determine NOAELs (mg/kg) in toxicity studies
2. Is there justification for extrapolating animal NOAELs to HED based on mg/kg (or other appropriate normalization?)

↓  ↓

NO YES

Convert each animal NOAEL to HED based on BSA

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

Select HED from most appropriate species

Choose safety factor and divide HED by that factor

Maximum Recommended Starting Dose (MRSD)

Consider lowering dose based on a variety of factors, e.g., PAD
Step 1: Determination of No Observed Adverse Effect Level (NOAEL)

NOAEL Definition
The highest dose level that does not produce a significant increase in adverse effects in comparison to the control group

Not the same as the no observed effect level

Review all available data in all species tested

Adverse events can be overt toxicities, surrogate laboratory markers, or exaggerated PD effects
Adverse effects defined as events that are considered unacceptable if produced by the initial dose in a Phase I clinical trial

FDA Guidance for Industry July 2005
Step 2: Convert Animal Dose to Human Equivalent Dose (HED)

Normalization of toxic dose levels across species often based upon body surface area
Deviations from BSA normalization must be justified

Animal dose in mg/kg is converted to mg/m2 and reconverted to mg/kg
Many cancer treatments are dosed based on BSA (mg/m2)

_FDA Guidance for Industry July 2005_
HED Calculation

HED (mg/kg) = __________ x Animal Dose (mg/kg)

Human Km

Km: mg/kg to mg/m^2 conversion factor
  Adult human = 37
  Child (20 kg) = 25
  Mouse = 3
  Rat = 6
  Cynomolgus, rhesus or stumptail monkey = 12

FDA Guidance for Industry July 2005
Exceptions to BSA Scaling

Weight based (mg/kg) scaling
- Oral therapies limited by local toxicities
- Exposure parameters that scale by weight predict toxicity
  - Example Cmax for antisense molecules
- Proteins administered IV with Mr > 100,000

Other scaling factors
- Alternate routes of administration (e.g. topical, intranasal, subcutaneous, intramuscular)
  - Normalize to area of application or to mg
- Administration into anatomical compartments with limited outside distribution (e.g. intrathecal, intravesical, intraocular, or intrapleural)
  - Normalize to compartmental volumes
Step 3: Most Appropriate Species Selection

After the NOAEL from all toxicology studies are converted to HED, then the MRSD must be derived from the most appropriate species.

By default, use the most sensitive species, but must also consider…

- Pharmacokinetic ADME differences
- Class pharmacodynamic effects
- Agent pharmacology, receptor cross reactivity, etc

Example

Phosphorothioate antisense DLT in humans and monkeys is complement activation

Does not occur in rodents

FDA Guidance for Industry July 2005
Step 4: Application of a Safety Factor

Applied to the HED derived from the NOAEL from the most appropriate species

Divide the HED by the safety factor to determine the MRSD

By default, a safety factor = 10 is recommended
    May raise or lower with justification
Altering the Safety Factor

Increasing the safety factor
  - Steep dose response curve
  - Severe toxicities anticipated
  - Non-monitorable toxicity
  - Toxicities without premonitory signs
  - Variable bioavailability
  - Irreversible toxicity
  - Unexplained mortality
  - Large PK variability
  - Non-linear PK
  - Inadequate dose-response
  - Novel therapeutic target
  - Animal models with limited utility

Decreasing the safety factor
  - Requires highest quality toxicology data
  - Well characterized class of drugs
  - If NOAEL is based on toxicity studies of longer duration than the proposed clinical trial
Step 5: Adjustments Based on the Pharmacologically Active Dose

If a robust estimate of the pharmacologically active dose (PAD) is available from preclinical studies

Convert to HED and compare to the MRSD

If PAD < MRSD consider decreasing the starting dose
A Phase I Study of TGN1412: A Critical Dissection of Clinical Disaster

A Failure of Preclinical Safety Testing?
CD28 and T Cell Activation

CD28 is a co-stimulatory receptor found on all CD4 regulatory T-cells and about 50% of CD8 cytotoxic T-cells. CD28 signaling is activated by endogenous membrane-bound ligands, B7-1 (CD80) and B7-2 (CD86).

Normal activation of T-lymphocytes requires two signals:
- **First Signal**: Specific antigen complex presented to the T-Cell receptor (TCR) by the antigen-presenting cell (APC).
- **Second Signal**: Co-stimulatory activation of CD28 on the T-cell by B7 molecules.

Graphic illustration

www.mpip.org/therapy/artcl5img2.gif
“Super Agonist” Anti-CD28 Antibodies Activate T-Lymphocytes

Directly activate T-cells via CD28 WITHOUT requiring TCR activation

Binds CD28 specifically in a linear conformation

T-cells activated independent of the T-cell receptor

Preferential activation of regulatory (CD4+) T-cell subsets
  TH1: activate WBC mediated immunity, and self vs. graft response
  TH2: stimulate B cells and antibody production

Graphic illustration of superagonistic → T-cell-activation
**Therapeutic Rationale**

Autoimmune diseases
Enhance regulatory T cells to block autoimmunity

Efficacy in preclinical models of rheumatoid arthritis, autoimmune neuritis, autoimmune encephalomyelitis

Hematological malignancies
Capacity to reconstitute collapsed T cell compartment in diseases such as B-CLL

Ex vivo evidence of activation of T cells independent of TCR specificity

Improve antigen presentation by B-CLL cells

Expansion of regulatory T lymphocytes and induction of anti-inflammatory cytokines

No detectable adverse side effects other than lymphocytosis
TGN1412: An Anti-CD28 “Super Agonist” Antibody

Recombinant, humanized IgG4-kappa antibody, MW 24 kDa
Developed by TeGenero, a European biotechnology company

Engineered from monoclonal mouse anti-human CD28
Expressed in CHO cells

Binds to human CD28 with Kd = 1.88 nM

Prepared in a buffered solution for IV infusion
TGN1412 Non-Clinical Safety Studies

Cross species amino acid homology of binding epitope on CD28
  Cynomolgus monkey (Macaca fascicularis) vs. human
    Identical binding epitope
  Rhesus monkey (Macaca mulatta) vs. human
    1 AA difference
  Marmoset monkey (Callitrix jacchus) vs. human
    2 to 6 AA differ
  Rodent vs. human
    Very low homology

Anti-rat CD28 orthologue mAb also developed and tested
  No substantial safety signals

In vitro treatment of human PBMC with soluble TGN1421
  Some polyclonal T cell proliferation
  Some T cell specific cytokine secretion
TGN1412 Primate Toxicology

TGN1412 long-term administration to Macaca mulatta
   No change in systemic cytokine serum conventions
   No long term (5 months) side effects

TGN1412 in cynomolgus monkeys expanded CD4+ and CD8+ T cells
   Activation of T cells peaked at day 15
   Mild lymphocytosis

Moderate elevation of IL-2, IL-5, IL-6 but no evidence of severe acute release of cytokines
   No evidence for cytokine storm
**TGN1412 Regulatory Oversight**

Initial first in human, first in class TGN1412 study proposed by sponsor

Approved by two European Regulatory Agencies (in UK and in Germany) and by local research ethics committee

TGN1412 starting dose calculation of 0.1 mg/kg met current regulatory requirements
TGN1412 Clinical Study Design

Sponsor
TeGenero

Contract Research Organization
Parexel International

TGN1412 Supplier/Manufacturer
Boehringer Ingelheim

Location
Parexel Clinical Pharmacology Unit housed in leased space at Northwick Park and St. Mark’s Hospital (UK NHS Hospital) in London

Suntharalingam et al NEJM 2006
TGN1412 Clinical Study Design

Research Subjects
Normal healthy paid volunteers
First cohort of 8 Subjects: 6 treatment and 2 controls
All males, median age 29.5 yr (19 to 34 yr) in good health

Randomized, double-blind, placebo-controlled
Planned admission on day 1 and to remain inpatient until day 3

Single 3-6 minute intravenous infusion within minutes of all subjects
All subjected treated 10 minutes apart

Dose: 0.1 mg/kg of TGN1412 infused at 2 mg/min
Other planned doses: 0.5, 2, 5.0 mg/kg

Suntharalingam et al NEJM 2006
TGN1412 Acute Reactions

Study initiated at 0800 hr on 13 March 2006
  Reactions started within 90 min

Rapid onset of clinical symptoms
  Headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension

Rapid induction of pro-inflammatory cytokines (cytokine storm)

At 12-16 hr became critically ill
  Pulmonary infiltrates, lung injury, renal failure, disseminated intravascular coagulation

*Suntharalingam et al* NEJM 2006
TGN1412 Immunological Changes

Profound lymphopenia and monocytopenia noted at 24 hours

Extreme elevations of
  TNF-alpha
  IL-2, IL-6, and IL-10
  Interferon-gamma

Prolonged (2 days) cytokine release in 2 most ill pts

Suntharalingam et al NEJM 2006
TGN1412 Critical Care

All 6 treated patients transferred to ICU at adjacent public hospital within hours
Two controls allowed to leave prior to breaking double blinded code

Critical care support initiated
  Hemodialysis, vasopressors, respiratory support, high dose steroids, anti-IL2 receptor antagonist antibodies

Two patients developed cardiovascular shock and acute respiratory distress syndrome requiring mechanical ventilation

Suntharalingam et al NEJM 2006
TGN1412 Patient Outcomes

All patients survived (miraculously)

Long-term neurological, psychological, and immunological sequelae to be defined

Suntharalingam et al NEJM 2006
What Went Wrong?

Extensive review by healthcare agencies and committees
EMEA
UK Medical and Healthcare Products Regulatory Agency (MHRA)
Expert Scientific Group on Phase One Clinical Trials

Clinical trial findings published in the NEJM
Suntharalingam et al NEJM 2006

Lessons are still being debated
TGN1421 Protocol Violations

Minor protocol violations found during retrospective scrutiny
  Documentation of full medical history for 1 subject was incomplete
  Minor employment procedural error
  Sponsor’s insurance policy not reviewed
  Placebo treated volunteers not formally unblinded before discharge
  TeGenero/Parexel contract not in place prior to study initiation
TGN1412 Aftermath

No errors in manufacture, formulation or administration

No contamination with bacterial endotoxin

Conclude that unpredicted biological effects of the test substance caused the dramatic clinical effects

TeGenero files for bankruptcy in June 2006
Failure of Non-clinical Safety Studies?

Preclinical in vitro studies failed to predict toxicity in vivo
mAb was not presented to lymphocytes in a manner that mimicked its presentation in vivo

Binding of TGN1412 to cell surfaces is a requirement for activation of lymphocytes and triggering of the cytokine storm

In vivo primate studies failed to predict human toxicity
Lymphocytes from Cynomolgus monkeys do not respond to TGN1412 binding in the same way as human cells
TGN1412 is not superagonistic in this species (a pharmacodynamic difference)

Stebbins et al, J Immunol 2007;179:3325
In Vitro Lymphocyte TGN1412 Studies (Stebbins et al, J Immunol 2007;179:3325)

Graphic illustration of Human PBMC + Aqueous TGN1412 → No proliferation or release of TNF-α, IL-6 or IL-8

and Primate PBMC + Air-dried TGN1412

Graphic illustration of human PBMC + Air-dried TGN1412 → Proliferation and release of TNF-α, IL-6 or IL-8

CYTOKINE STORM!
**TGN1421 Trial Learning Points**  
*(modified from Dayan et al, Br J Immunol 151:231)*

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<tr>
<th>TGN1412 Study Problem</th>
<th>Detail</th>
<th>Learning Point</th>
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<tbody>
<tr>
<td>Interpretation of preclinical studies</td>
<td>Low level cytokine release in primates should have prompted more caution</td>
<td>Minor but potentially important effects in preclinical studies should raise caution across species</td>
</tr>
<tr>
<td>Use of human in vitro studies</td>
<td>Insufficient in vitro human studies on PBL were performed</td>
<td>In vitro studies on human material as close as possible to the target tissue can be important</td>
</tr>
<tr>
<td>Location of study unit</td>
<td>Located in a tertiary care hospital</td>
<td>Rapid access to an intensive care unit was important as events unfolded rapidly</td>
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## TGN1421 Trial Learning Points
*(modified from Dayan et al, Br J Immunol 151:231)*

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<tr>
<td>Choice of starting dose</td>
<td>Subtle difference between primate and human target may explain marked difference in potency. Calculation of initial dose based on NOAEL proved to be dangerously wrong</td>
<td>Prediction of risk and dose range from animal studies may prove unreliable: extra caution with wider margins of safety are required with potentially risky modes of action. Use of MABEL?</td>
</tr>
<tr>
<td>Dosing interval between subjects</td>
<td>No proper interval allowing for the observation of possible side effects between subjects</td>
<td>In FIH studies, investigators should expect the unexpected</td>
</tr>
<tr>
<td>Preparation for adverse events</td>
<td>Preparation for possible adverse events (cytokine storm) was inadequate. Investigators did not expect it, recognize it, or treat it early</td>
<td>Where there is a known theoretical risk, investigators should plan for its potential occurrence</td>
</tr>
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**MABEL Instead of NOAEL, MAYBE?**

Re-evaluation of the TGN1412 trial has led to new recommendations for starting dose selection in Europe

EMEA Guidelines, 2007

Consider factors that may add to potential risk
- Mode of action
- Nature of target
- Relevance of animal models

MABEL: minimal anticipated biological effect level
- The anticipated dose level leading to a minimal biological effect level in humans
- Consider differences in sensitivity for the mode of action across species

Consider selection of starting doses based upon reduction from the MABEL, not NOAEL dose
Calculation of MABEL
(EMEA Guidelines, 2007)

MABEL calculations should utilize all in vitro and in vivo information from PK/PD experiments, including…

- Target binding and receptor occupancy data in target cells in vitro in human and animals

- Concentration-response curves in vitro in target human cells and dose/exposure-response in vivo in relevant animals

- Exposures at pharmacological doses in relevant animals

Wherever possible an integrated PK/PD modeling approach should be used

Apply a safety factor to the MABEL for the recommended starting dose

If NOAEL method gives a different estimation, use the lowest value unless otherwise justified
Problems with the MABEL (or any approach)

Estimation of MABEL may prove difficult with some agents, such as those that target the immune system.

In vivo immune response are much greater than in vitro

Agents such as TGN1421 may act via a trigger or threshold effect.

Immunological cascade may amplify any biological action.

MABEL may not exist.

For other agents, overestimation of MABEL may lead to extremely low starting doses resulting in a conclusion of no biological activity.

*Dayan et al, Br J Immunol 151:231*
Issues Raised by TGN1412

Ethics of FIH trials in volunteers/patients

Species-specific pharmacology & toxicology of targeted agents

Immunologics/biologics offer special problems in evaluation

Greater transparency and input in early therapeutic development

Inherent risks in developing novel agents with new mechanisms of action
The Clinical Pharmacology Challenge!

Preclinical Pharmacology → Clinical Pharmacist → Early Clinical Trials

Traditional animal studies
PK/PD
Toxicology

Biomarkers & Molecular targets

Translational dose And toxicity endpoints

Traditional PK/PD Biomarkers & Molecular endpoints Patient selection

TRANSLATIONAL MEDICINE