Regulatory History, Challenges and Progress in Developing Biologics

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Overall Goal of Safety Assessment

To perform necessary preclinical animal testing to support clinical trials and marketing.

Definition of Biopharmaceuticals

- Products derived from characterized cells including bacteria, yeast, insect, plant, and mammalian cells.
- Includes proteins, peptides, their derivatives or products of which they are components.
- Examples include: cytokines, proteins, growth factors, fusion proteins, enzymes, receptors, hormones, and monoclonal antibodies.

Biotechnology Products

- The product is the process
- ADME different than traditional SM
- Large molecules (>500 Daltons)
- Genetic Engineering
- More than half of the molecules in development are “Biotech”
- Annual sales: >30 billion with over 150 molecules approved for marketing

Different Characteristics

Biologics (Large Molecules)[8]

- Immunogenicity
  - Neutralizes pharm. activity
- Case-by-case nonclinical studies
  - On-target toxicity, exag. pharm.
- Species specificity
- Route of administration
  - Parenteral (IV, SC)
- Metabolism
  - Proteolytic degradation
- Half-life
  - Longer (↓'s dosing frequency)
- Cross-reactivity
  - Possible homology; expression at multiple sites

≠

Drugs (Small Molecules)[8]

- No immunogenicity
- Routine nonclinical studies
- Non-specific, off-target toxicity
  - e.g., Cytotoxic agents target rapidly dividing cells
- Active across species
- Route of Administration
  - Oral (or IV)
- Degradation metabolites may induce toxicity
- Half-life
  - Shorter (↑'s dosing frequency)
- Safety margins higher

[8]Cosenza, ME; Safety Assessment of Biotechnology-Derived Therapeutics. Pharmaceutical Sciences Encyclopedia: Drug Discovery, Development, and Manufacturing. John Wiley & Sons, Inc. 2010.

Conventional/Small Molecule Toxicology Programs Well-Defined: Specific Guidance Exists

- Small molecule drugs tend to be less specific in their activity
- "Off-target" toxicities predominate and have driven expectations regarding toxicology studies
- Large historical database for small molecules
- Well-defined global regulatory standards
  - Multiple guidance documents available (ICH/FDA/ etc.)
  - "Check-box" like application of guidance

Regulatory standards for Biotechnology products have generally been flexible, developed on a case-by-case basis and more science driven than with traditional small molecules.

Historical Perspective of Biological Regulations

- 1902 Biologic Control Act/1906 Pure Food Drug Act
- 1972-Transfer of Biologics Regulation to FDA's Bureau of Biologics (prior regulated by NIH)
- 1982-Bureau of Drug and Biologics Merged
- 1987-Center for Biologics Separated from Center for Drugs
- 1993 Center for Biologics Re-organization into Review Divisions oriented toward product type
- 1995 REGO-Biologics Regulations Brought into Line with Drug Regulations
- 2003 CBER's incorporation of therapeutic proteins into CDER
- 2005 full integration within CDER review divisions

Two Main Laws

- **1938: Federal Food, Drug and Cosmetic Act (FD&C Act)**[3]
  - **Definition of "drugs" → inclusive of biologics**
  - **Federal government seizure of adulterated/misbranded drugs**
  - **Pre-market demonstration of safety required**

- **1944: Public Health Service Act (PHS Act)**[3]
  - **Biologics License Application (BLA)**
  - **Biologics required to be safe, pure, and potent, and manufactured in a license facility (CGMP)**
  - **Biologics subject to regulations under both FD&C and PHS Acts**

- **"Dual Licensing"**

[3]Scott, Steven R; Brady Robert P; Chung, Ellen Y (Edited by Mark Mathieu). Biologics Development: A Regulatory Overview (Chapter 1: What is a Biologic?). 3rd Edition. Waltham, MA: PAREXEL International Corporation, 2004.

Biotechnology Industry Growth

- 1980 – 5 INDs
- 1990 – 200 INDs

- Industry growth mirrored by CBER growth

- 2002 - Recognition that Biotech is part of mainstream medicine

2003 CBER → CDER

Review and Cultural Changes[4]

- ↓ Regulatory flexibility
- ↓ Open communication with reviewers
- ↑ Formalized communication
- ↓ Individualized approaches to clinical development
- ↑ Rigor of randomized trials
- ↑ Number of preclinical studies
- ↑ Size of overall safety databases
- ↑ Conservative approaches to clinical pharmacology

[4]Schwieterman, WD. Regulating biopharmaceuticals under CDER versus CBER: an insider's perspective. Drug Discovery Today. 11(19/20)2006.

First in class biologics likely to be regulated more strictly[15]

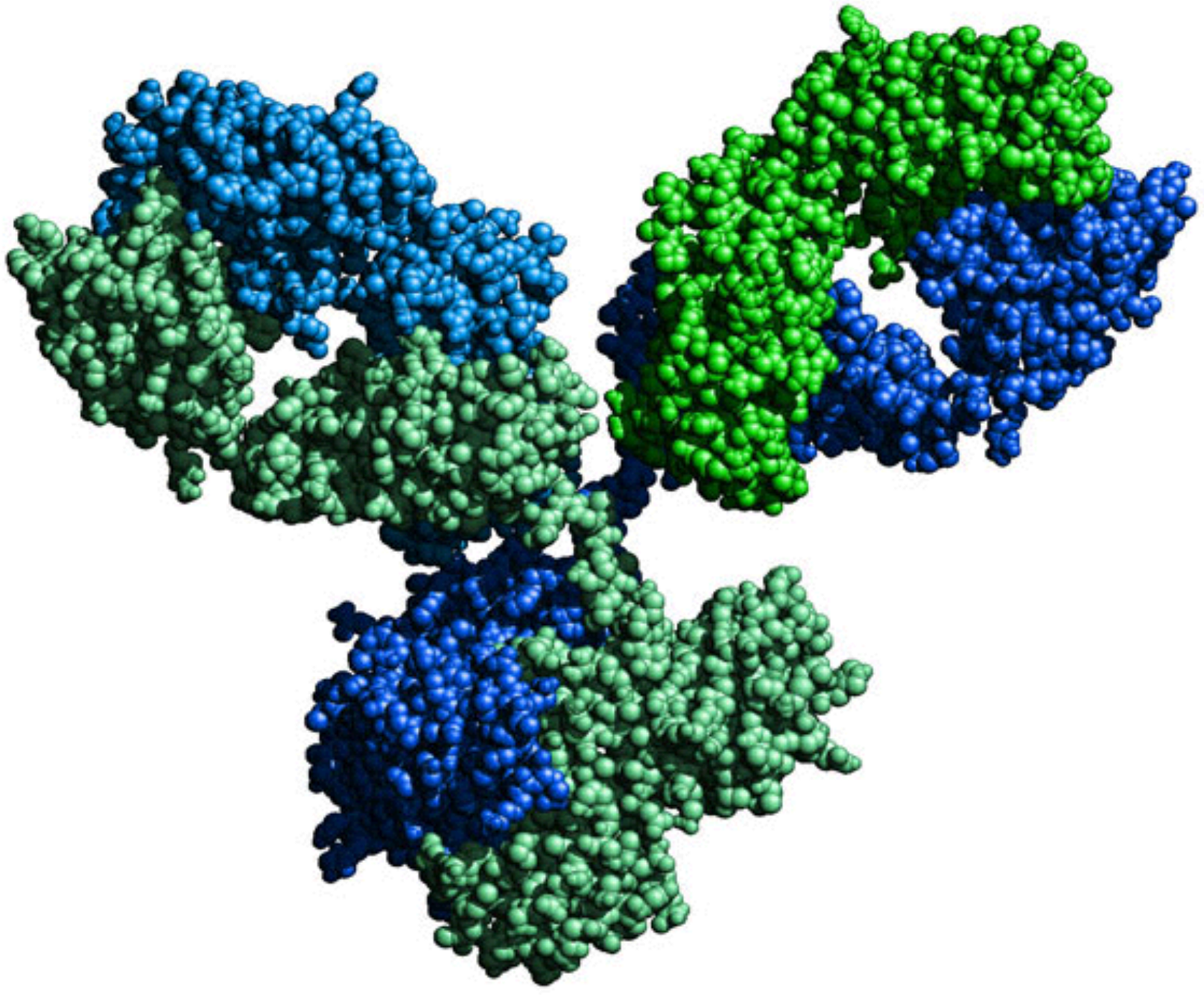
- 1995-2007: 174 approved (136 US, 105 EU, 67 both regions)
  - 82 safety-related regulatory actions for 41 biologics (23.6%)
  - Higher probability of safety event compared with later approved products (HR, 3.7; 95% CI, 1.5-9.5)

General disorders and administration site conditions (26.8%), infections (22%), immune system disorders (15.9%), neoplasms - benign, malignant, and unspecified (12.2%)

[15]Giezen, TJ; Mantel-Teeuwisse, AK; Straus, SMJM, Schellekens, H; Leufkens, HGM; Egberts, ACG. Safety-Related Regulatory Action s for Biological Approved in the United States and the European Union. 2008. 30(16);1887-1896.

Biologics (Large Molecules)

Drugs (Small Molecules)

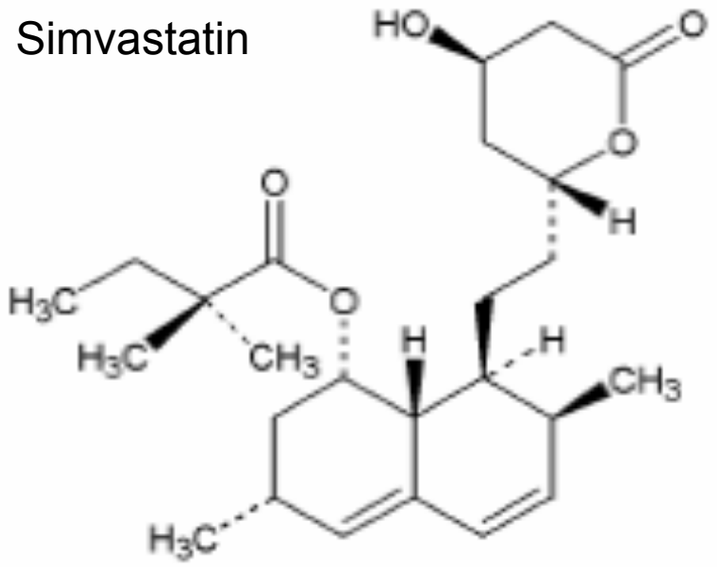


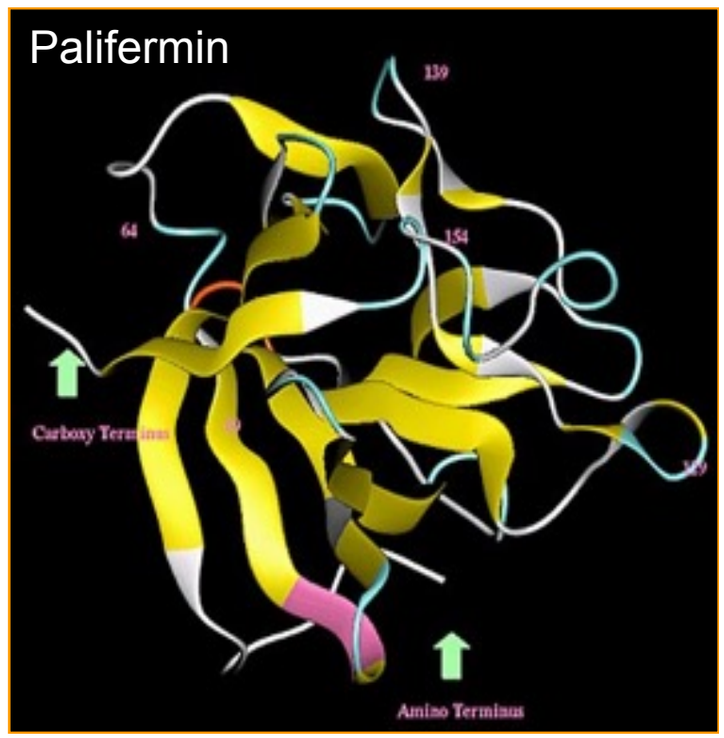
Conventional Drugs vs. Biologics: "Small Molecules" and "Large Molecules"

Drug	MW (daltons)
Conventional (Small Molecule, < 1000 MW)	
Zocor (simvastatin)	418.6
Prozac (fluoxetine)	345.8
Norvasc (amlodipine besylate)	567.1
Viagra (sildenafil citrate)	666.7
Sensipar (cinacalcet HCl)	393.9
Biopharmaceutical (Large Molecule, > 1000 MW)	
Epogen (epoetin alfa) – 167 aa glycoprotein	30400
Neupogen (filgrastim) – 175 aa protein	18800
Aranesp (darbepoetin alfa) – 165 aa glycoprotein	37000
Vectibix (panitumumab) – >1000 aa MAb	147000
Kepivance (palifermin) – 140 aa protein	16300
Neulasta (pegfilgrastim) – 175 aa pegylated protein	39000
Enbrel (etanercept) – 934 aa fusion protein	150000
Kineret (anakinra) – 153 aa protein	17300
Nplate (romiplastin) – >500 aa Fc fusion protein	60000
Prolia (denosumab) -- >1000 aa MAb	147000

Biologics Typically More Structurally Complex than Small Molecules

- Biologics are comprised of strings of amino acids
- Proteins have complex higher order structure
- Manufactured in living cells
- Undergo post-translational modification (eg. glycosylation and pegylation) and aggregation





List of Toxicology Studies for Protein Development

- Range Finding Studies
- 1 Month Studies
- 3/6 Month Studies
- Safety Pharmacology
- Reproductive Studies
- Tissue Cross-reactivity Studies (MoAbs)
- Irritation / Tolerance
- Others As Needed

Total Cost: $3 – 3.5 Million
Linear Time: 2 - 2.5 Years

List of Toxicology Studies for Small Molecule Development

- Screening Studies
- Range Finding Studies
- Acute GLP Studies
- 1 Month Studies
- Safety Pharmacology
- Mutagenicity Studies
- 3 Month Studies
- Reproductive Studies
- 6 Month Rat
- 1 Year Dog / Monkey
- Industrial Toxicology
- Diet RF Studies
- Carcinogenicity Studies

Total Cost: $5.5 – 7 Million
Linear Time: 4.5 - 5 Years

Toxicology Issues

Purpose of Animal Toxicity Studies To identify potential human toxicities

- To identify potential human toxicities
- To design specific animal tests to further define a toxicity or its mechanism
- To suggest specific toxicities to be monitored during clinical trials (e.g. hearing loss, neurotoxicity, Q-T prolongation, hematology changes)

Study Design Issues

- Species Selection
- Dose, route, and Frequency
- Length of Study
- Antibody Response
- Immunotoxicity

Selection of Animal Species

- Pharmacologically relevant
- Homology between species
- Disease models
- Transgenic animal
- Homologous proteins
- Species specific findings

Common Challenge for Biopharmaceuticals: Species Selection

- "Safety evaluation programs should include the use of relevant species" (ICH S6)

- One in which the drug is pharmacologically active due to expression of the target receptor/epitope

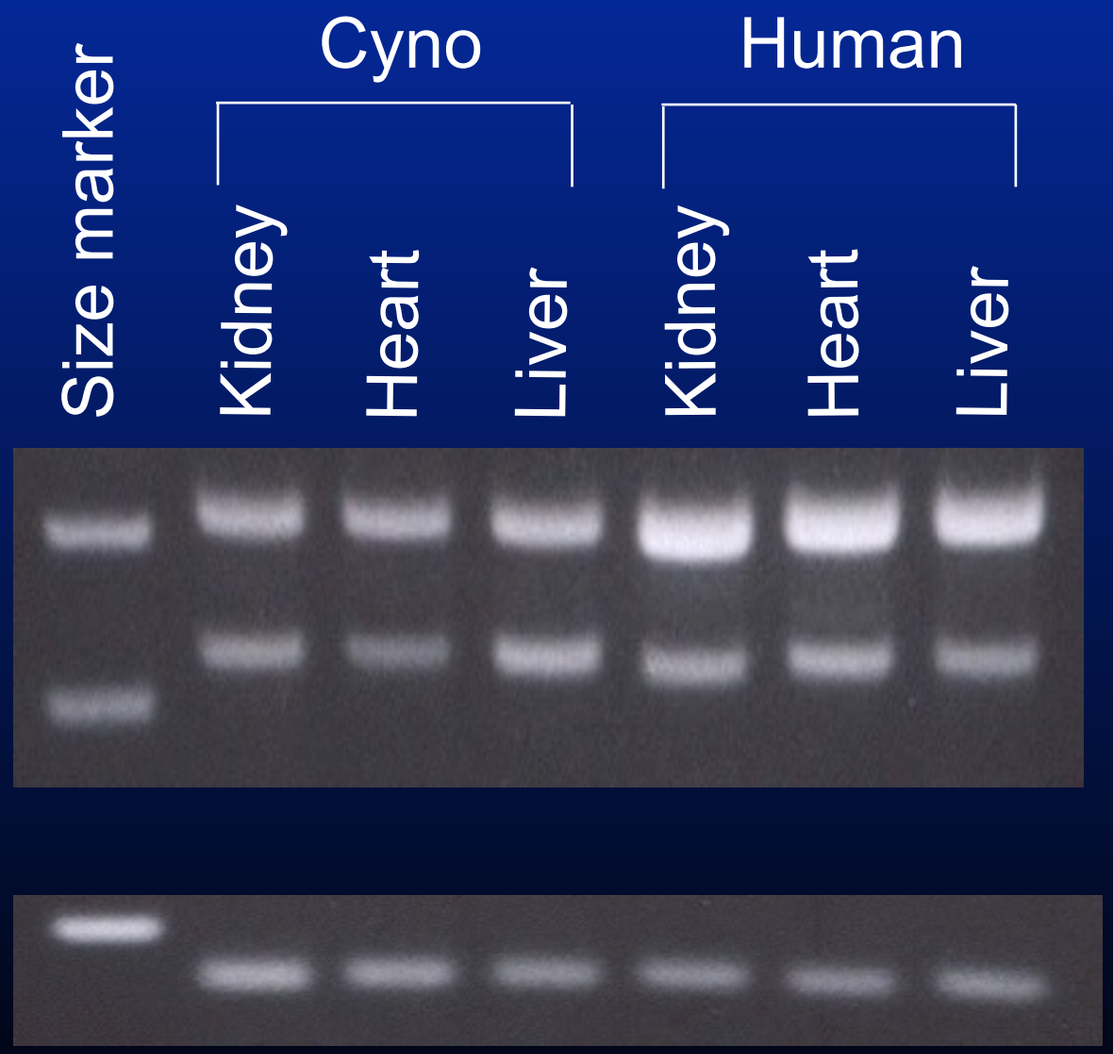
- Identification of relevant species can be challenging due to the high degree of specificity of biologic therapeutics

Species Selection Must be Justified: Types of Supportive Data

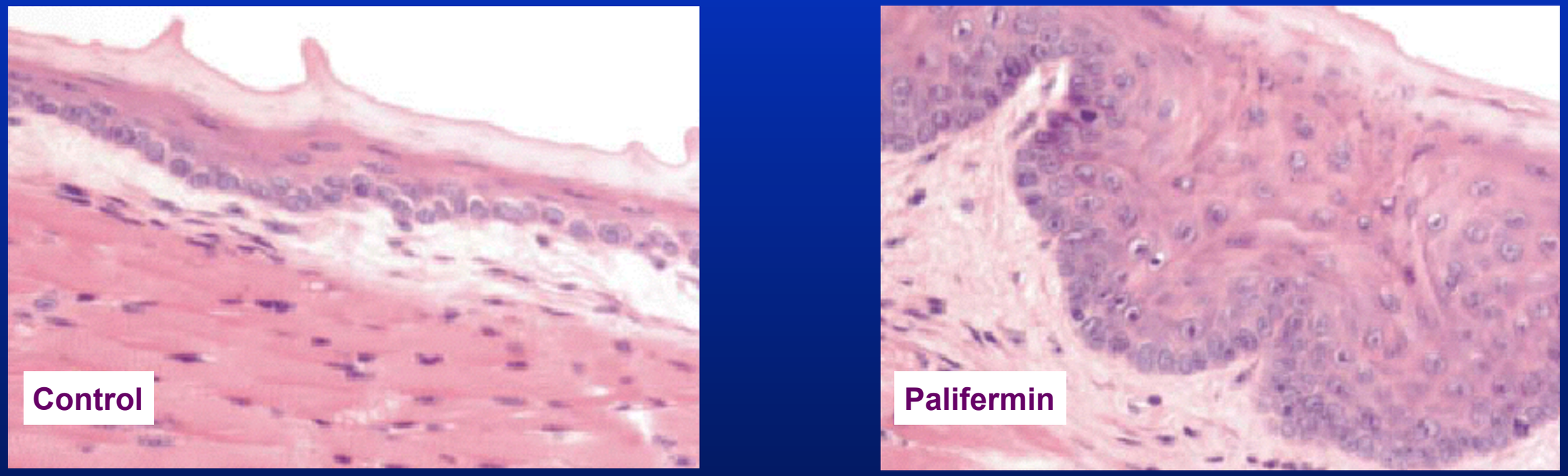
- Homologous target sequence between human and animal
- Comparable target/receptor expression & distribution in animal and human
- Comparable in vitro binding affinity of drug to human and animal target
- Comparable receptor/ligand occupancy and kinetics
- Comparable function of drug in human and animal cells in in vitro assay
  - Show intended neutralization or activation of target (eg, cell proliferation assay)
- Comparable in vivo pharmacological activity

Demonstration of Comparable MAb Target Expression: Cyno vs. Human

Expression pattern of epitope is similar in humans and cynos by RT-PCR



Demonstration of Comparable In Vivo Pharmacological Activity in Humans and Animals



Ventral tongue epithelium in mice treated with vehicle or palifermin for 3 days

- Kepivance→(Palifermin) protects the oral mucosa from chemo-/ radiotherapy induced damage in humans
- The drug increases epithelial cell proliferation in buccal biopsies
- Toxicology findings with palifermin largely limited to widespread epithelial thickening in rats and monkeys
  - *Exaggerated pharmacology*

[1] Potten CS, et al. *Cell Proliferation* 2002;35: (Suppl 1):22-31.
[2] Farrell CL, et al. *Cancer Res.* 1998;58:933-939.
[3] Farrell CL, et al. *Int J Radiat Biol* 1999;75:609-620.

Surrogates

- Alternative if molecule does not cross-react with other species
- Use in reproductive toxicology studies to spare use of primates and increase “N”
- Challenges of developing a surrogate
  - Cost
  - Time
  - Delay to other projects

Surrogate Molecules = Species Specific Analogous Products

- A surrogate is a molecule that "hits" the same target as the human drug in another species
  - Example: A fully human anti-human TNF mAb doesn't recognize mouse TNF; a surrogate could be a mouse anti-mouse TNF mAb
- Frequently useful for demonstrating proof of concept in preclinical pharmacology studies
  - Used less frequently for preclinical safety studies
- The suitability of the molecule as a surrogate of the human needs to be carefully evaluated
  - Identity, purity, stability, activity (binding/function), pharmacological mechanism, PK, immunogenicity, etc.

Dose Selection for Biologic Toxicology Studies

- For conventional drugs, dose selection is driven by expectations for a toxic and a no-effect dose
  - Same goal generally applies to biologics (ICH S6); however, for many biologics, there is little or no toxicity
- In these cases, scientific justification of dose selection rationale is required
- High Dose based on PK and PD
  - **Dose that gives maximum pharmacological activity in preclinical species (e.g. saturation)**
  - **Dose that gives an up to 10-fold exposure multiple over maximum anticipated clinical dose/exposure**
- Need to account for species differences in target binding and in vitro pharmacological activity
- If no toxicity, then additional toxicity studies at higher multiples are unlikely to provide additional useful info (S6 R1)

Single and Repeated Dose Toxicity Studies

- **Single dose studies**
  - For small molecules, used to evaluate acute toxicity
  - Acute effects generally not seen with biologics
  - **May** be useful for dose-ranging for repeated dose studies
  - *Rarely conducted as part of biologic toxicology packages*
- **Repeated dose studies**
  - Define spectrum of pharm/tox effects and allow clinical dose recommendations
  - Include assessments of toxicokinetics, *immunogenicity (S6 R1)*, and **local tolerance** (at injection site)
  - Include assessments of recovery (S6 R1)
  - Careful consideration of route, regimen, and duration

Dosing Route/Regimen of Toxicology Studies with Biopharmaceuticals

- The route and dosing regimen should reflect the intended clinical use or exposure (ICH S6)
  - Example
    - Once-weekly IV for 6 weeks in the clinical study
    - Once-weekly IV for 6 weeks in the supportive toxicology study, if PK parameters (peak, trough, half-life) are equivalent
      - *Dosing frequency can be adjusted for differing PK*
- Absolutely critical to characterize PK of your molecule in your toxicology species to inform the correct dosing paradigm for pivotal studies
  - This is particularly key for biologics, where intermittent dosing (instead of daily) is typical due to generally longer half lives

Duration of Dosing in Repeated Dose Toxicology Studies

- The duration of repeated dose studies should be based on the intended duration of clinical exposure and disease indication (ICH S6)
  - The longest duration of animal dosing is *generally* 1-3 months for most biologics
  - For drugs intended for short-term use (e.g. ≤ 7 days) and for acute life-threatening diseases, studies of 2 weeks duration have been sufficient
- For chronic use products, the adequacy of 6-month chronic studies is supported by the scientific experience with biopharmaceuticals to date (S6 R1)
- Studies of longer duration are not anticipated to provide useful information to change the clinical course of development (S6 R1)

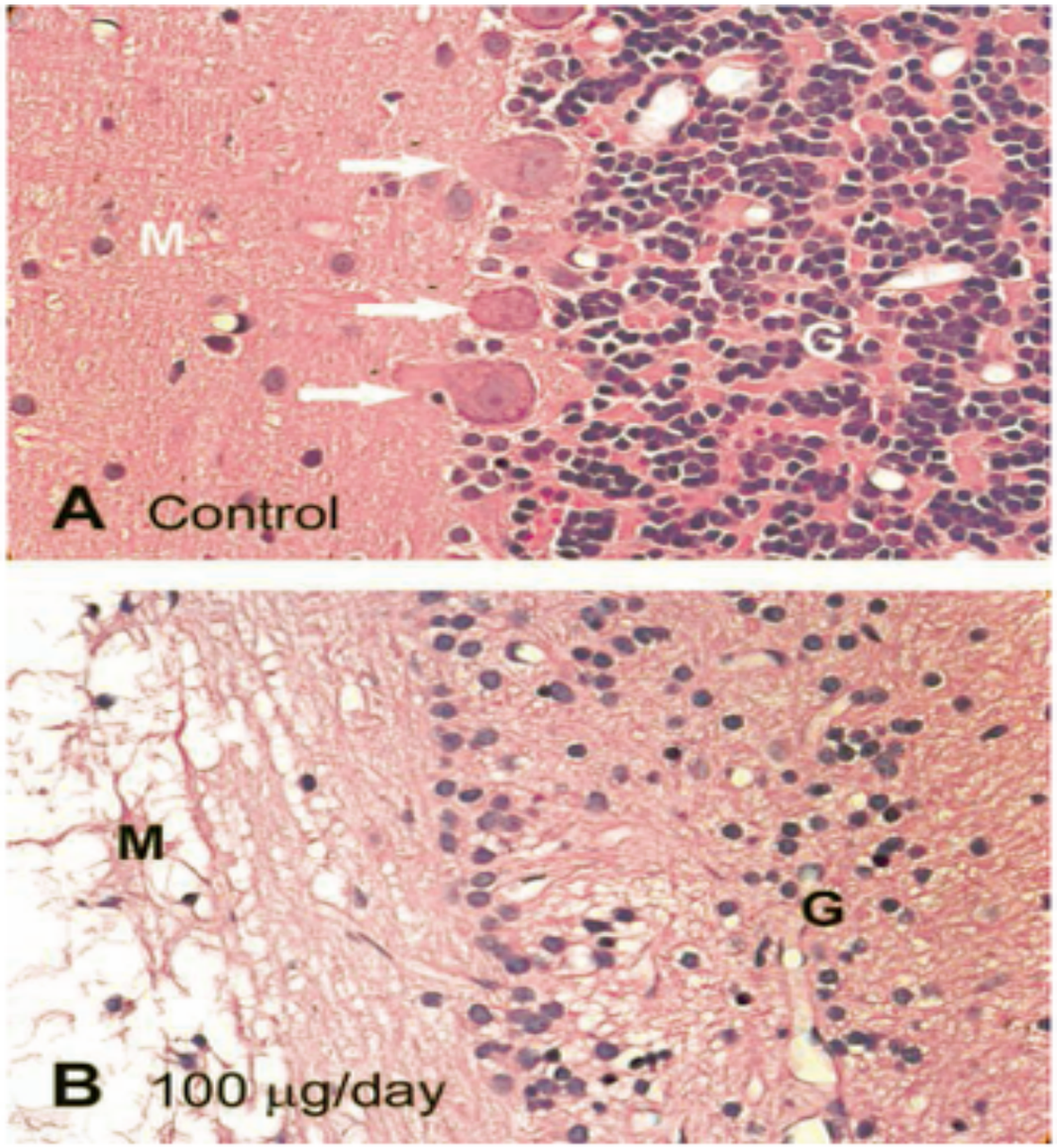
Recovery Periods on Repeated Dose Toxicity Studies (ICH S6)

- Recovery of pharmacological and toxicological effects with potential adverse clinical impact should be understood (S6 R1)
- This information can be obtained by including a non dosing period in at least 1 study
  - *Complete recovery is not considered essential*
- If no adverse effects at the end of treatment period, no need for recovery assessment
- Not intended to assess delayed toxicity or immunogenicity
- Challenges of defining recovery periods for biopharmaceuticals
  - Prolonged PD effects and persistence of drug

r-metHuGDNF Example: Incidence of Effect Greater at End of Recovery Period

TABLE 4.—Incidence of cerebellar lesion at scheduled necropsies

Dose (μg/day)	End of Treatment Period Necropsy		End of Recovery Period Necropsy	
	Males	Females	Males	Females
0	0/5	0/6	0/2	0/2
15	0/4	0/6	0/2	0/2
30	0/4	0/5	0/3	0/3
100	1/4	0/6	1/2	2/3



In this case, the lesion was originally noted in recovery animals and only seen in end of treatment animals upon retrospective review.

Immunogenicity

- **"Most biotechnology-derived pharmaceuticals intended for humans are immunogenic in animals." ICH S6**
- **Antibodies must be measured and characterized**
- **Effect on Pharmacokinetics**
- **Effect on Pharmacodynamics**

Clinically Relevant Antibodies

- Clearing Antibodies
- Sustaining Antibodies
- Neutralizing Antibodies
- Antibodies that cross-react with endogenous protein

Tissue Cross-Reactivity Study (for mAb)

- Cryosections of ~37 tissues from humans and animal species
- Drug (mAb) is labeled for staining and applied to sections
  - Sections are evaluated microscopically for <u>expected</u> and <u>unexpected</u> binding
- Unexpected binding could be suggestive of potential concern
  - *Current regulatory standards expect study to be conducted prior to phase 1 (PTC MAb 1997)*
  - *Used to be used for toxicology species selection – No longer believed to be appropriate (*S6 R1)

Genotoxicity

For many older biotechnology products a standard genotoxicity package (Ames, CHO / HGPRT, in vitro chromosomal aberration assay, mouse micronucleus assay) was performed. Today these are not usually performed unless there is a concern with a contaminant, impurity, organic linker, or conjugate.

Carcinogenicity Studies

- ICH S6: Case by Case (generally inappropriate)
- May depend on duration of clinical use and patient population
- ICH S1A: Generally not needed for endogenous substances….replacement therapy
- CPMP: required for insulin analogues

Carcinogenicity Studies: Standard Paradigm for Small Molecules (ICH S1A, S1B)

- The objectives are to identify tumorigenic potential in animals and to assess the relevant risk in humans
- Generally required for drugs intended to be used for ≥ 6 months in humans (or if cause for concern)
- Carcinogenicity assays conducted in 2 species
  - 2-year bioassay in rats
  - 2-year (or short-term alternative) bioassay in mice

- For biologics, species/target specificity and immunogenic potential generally make application of standard paradigm inappropriate

Carcinogenicity Studies Generally Not Needed for Biopharmaceuticals (ICH S6)

- Standard carcinogenicity bioassays are generally inappropriate
- Product specific assessment of carcinogenic potential may still be needed depending on duration of clinical dosing, patient population and/or biological activity of the product (e.g. growth factors, immunosuppressive agents, etc.)
- Potential to influence tumor life cycles may demand alternative studies

Kepivance (rHuKGF or Palifermin): Used Clinically to Protect Oral Mucosa from Chemo-/Radiotherapy Induced Damage

- Drug's intended therapeutic effects involve a proliferative influence on epithelium
  - Receptor (KGFR) expressed by epithelial cells
- Solid tumors of epithelial origin may express the receptor
- Theoretically, treatment with the drug to protect normal epithelial tissues could adversely impact tumors that express the receptor
- ***Drug does not interact with DNA, and would not be expected to induce tumors***
  - ***Typical carcinogenicity study wouldn't address risk***
- ***How do you address concerns?***

“Consideration” of Carcinogenic Potential for Kepivance

- √ KGFR expression characterization of human solid tumor cell lines
- √ In vitro cell growth assays to assess potential of Kepivance to influence growth rate of KGFR+ tumor cell lines
- √ In vivo xenograft studies (in nude mice) to assess potential of Kepivance to influence growth of KGFR+ tumor cell lines
- √ In vivo transgenic rasH2 (Tg.rasH2) mouse model to assess the drug’s potential to influence the rate of spontaneous tumor development

Kepivance→ U.S. Package Insert

- 5 WARNINGS AND PRECAUTIONS
  - 5.1 Potential for Stimulation of Tumor Growth
  - …Kepivance has been shown to enhance the growth of human epithelial tumor cell lines in vitro and to increase the rate of tumor cell line growth in a human carcinoma xenograft model (see Clinical Pharmacology (12.1)).
- 13 NON CLINICAL TOXICOLOGY
  - 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
  - Carcinogenicity
    - No treatment-related increase in the incidence of neoplastic lesions occurred in transgenic rasH2 mice treated with 9 weekly intravenous doses of palifermin, at 167-fold higher than the recommended human dose (on a mcg/kg basis).

Early Biotech Products were usually replacement molecules (Human Insulin).

Newer, more complicated molecules have led to more sophisticated approach to safety assessment.

Before ICH safety assessment plans were often regional and less science based than today

- Focus was on contaminants.
- Assumption was that toxicity was related to pharmacology only.

ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (1997)[9]

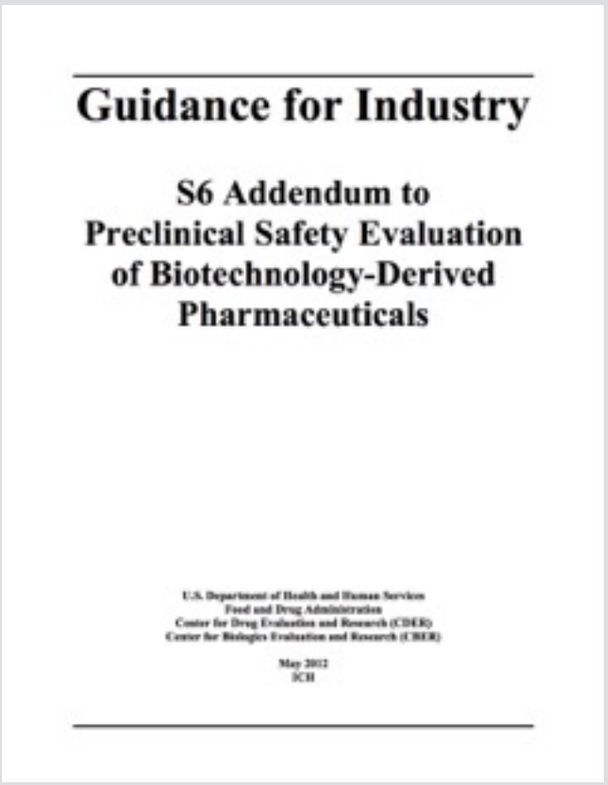
- **Concepts applicable to both biologics and drugs**
  - What are the known or anticipated risks?
  - Toxicities monitorable, reversible, clinically manageable?
  - What is the initial safe starting dose and dose limiting toxicities?

- **ICH S6 – Case by case approaches for biologics**[10,11]
  - Pharmacologically relevant vs. non-relevant species
  - Animal models of disease
  - Immunogenicity testing and implications
  - Genotoxicity, chronic toxicity testing, tissue cross-reactivity, carcinogenicity testing and preclinical study design
  - Consistent with 3R's (reduction, refinement, replacement)

[9]International Conference on Harmonization S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, July 1997
[10]**Anne M. Pilaro, Ph.D. FDA/CDER/ODE VI/DTBOP.** *Nonclinical Safety Testing for Biological Therapeutics for Cancer Treatment.* **Working with FDA: Biological Products and Clinical Development. NCI BRB Workshops. Accessed at: http://web.ncifcrf.gov/research/brb/newsEvents.aspx . Accessed: August 2011.**
[11]**Anne M. Pilaro, Ph.D. FDA/CDER/ODE VI/DTBOP. Pharmacology and Toxicology Information in Support of Protein Therapeutics. NCI Small Business Initiative. Workshop on Clinical Development. June 24, 2004**

ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals

- ICH S6 (1997) is key guidance
- Describes a flexible, case-by-case, science-based approach to biopharmaceutical preclinical safety evaluation
- Addendum to S6 (R1), May 2012
  - Clarification/update to S6 content, reflecting scientific advances and experience gained since original publication



ICH S6 Issues

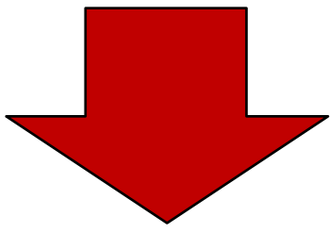
- **Final addendum released May 2012!**
  - Species Selection (# and use of homologous proteins)
  - Study Design (length of study duration and recovery)
  - Immunogenicity
  - Reproductive and Developmental Toxicity (↓ unnecessary repro toxicology studies)
  - Carcinogenicity

TeGenero

2006 - Phase 1 Study (6:2 healthy volunteers)

Anti-CD28 monoclonal antibody (fully humanized) [19]

- Binds CD28, directly activates T-lymphocytes causing T-cell immune response (i.e., “Superagonist”)
  - Bypasses 2-signal T-cell activation



Cytokine Release Syndrome (CRS)
induced by massive T cell activation that lacked antigenic specificity and indiscriminately attack normal tissues→ exaggerated pharmacology → organ failure

⇒ Nonclinical safety requirements met (rat, cynos), and no product quality issues⇐

BUT

⇒ Safe dose not established – Animal models clearly not relevant ⇐

[19] Horvath, C.J.; Milton, M.N. The TeGenero Incident and the Duff report Conclusions: A Series of Unfortunate Events or an Avoidable Event? Toxicologic Pathology. 37:372-383. 2009.

FDA Response

No major FDA policy shifts

→ More focus of reviews on justification of starting doses, relevance of animals models, dosing intervals

→ EMA has issued a guideline on strategies to identify/mitigate risks in FIH trials with investigational products

→ Good practice to consider principles in this document for US IND Applications

Duff Report

22 Recommendations…some specific to biologics[19]

- Reemphasized ICH S6 - case-by-case, science based approach
- Special considerations for new agents where primary pharmacological action cannot be demonstrated in an animal model
- When likely that preclinical models may be poor guide to human responses → factor into starting doses
- More communication strongly recommended between developers and regulatory authorities at an earlier stage (especially for higher risk agents)

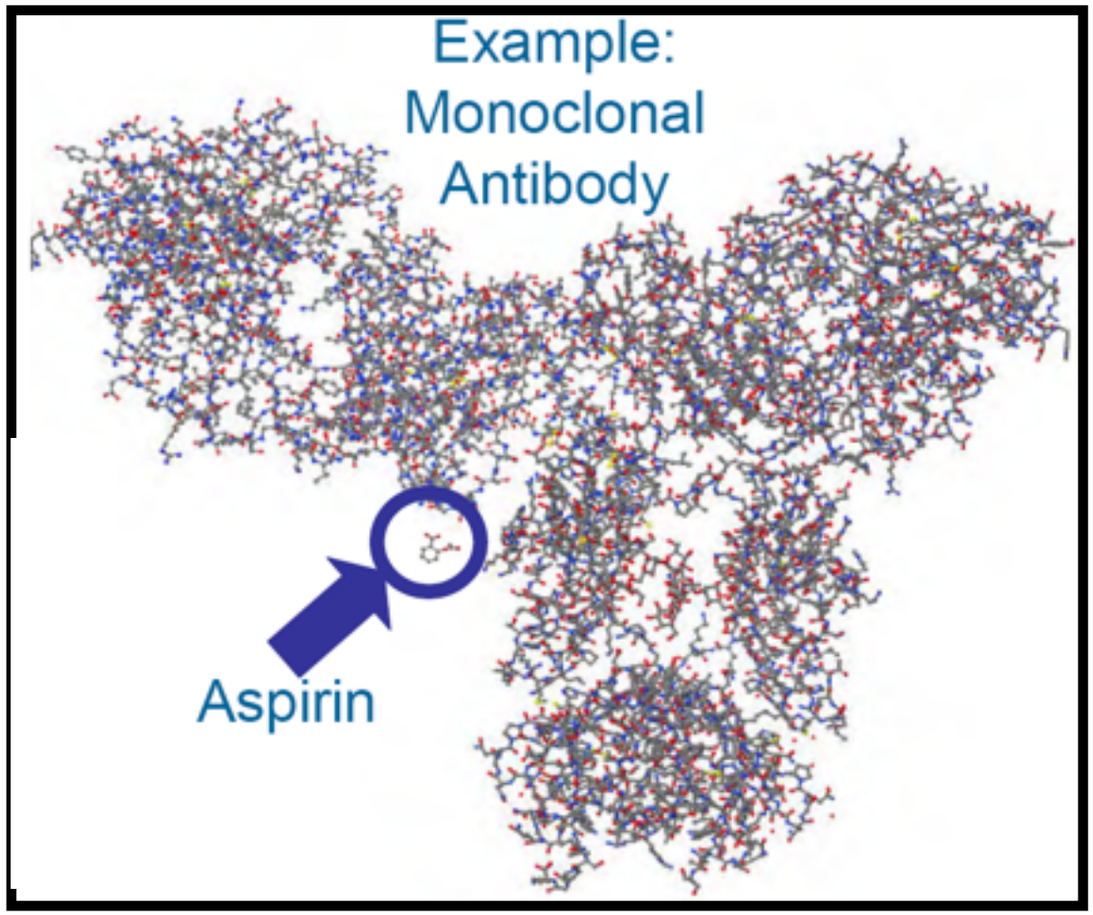
[19]Horvath, C.J.; Milton, M.N. The TeGenero Incident and the Duff report Conclusions: A Series of Unofortuniate Events or an Avoidable Event? Toxicologic Pathology. 37:372-383. 2009.

Biosimilars

- Biosimilars
  - EU legislation (2005) – “Similar Biological Medicinal Product”
  - WHO guidelines (2010) on “Similar Biotherapeutic Products (SBPs)”
  - US Biologics Price Competition and Innovation Act (BPCI Act) in 2009
    - Established an approval pathway for “highly similar” or “interchangeable” biological products
    - BPCI signed into law in 2010 as part of Patient Protection and Affordable Care Act (PPACA)
  - FDA tasked with implementation -- Several guidances have been released and more to come.
    - Scientific Considerations in Demonstrating Biosimilarity to a Reference Product: Draft February 2012

Key Concepts/Issues Related to Biosimilars

- Impossible to make an identical copy of a biologic
- "Highly similar" standard
- Interchangeability/ substitutability
- Naming conventions
- Extrapolation across indications
- Data exclusivity



http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM239634.pdf

Comparison of Data Packages for Authorization

Data	Innovator Product (small molecule or biologic)	Generic Medicine	Biosimilar
Quality/CMC (analytical)	**Full package**	**Full "standalone" package for proposed generic** **Comprehensive comparative studies with RP**	**Full "standalone" package for proposed biosimilar** **Comprehensive comparative studies with RP**
Nonclinical	**Full package** •Pharmacology •PK •Toxicology	**No data required**	**Abbreviated package (pending on quality data)** •Pharmacology •PK •Toxicology
Clinical	**Full package** •Phase 1 •Phase 2 •Phase 3 (**all** indications) •Post-approval commitments •Risk management plan	**Abbreviated package** •Bioequivalence study (often normal volunteers) •No Phase 1 •No Phase 2 •No Phase 3	**Abbreviated package (pending on quality data)** •Phase 1 (PK/PD) •No Phase 2 •Phase 3 (efficacy and safety) **•May get extrapolation of indications** •Risk Management Plan

B. Mounho

A few other Regulatory Pathways to Consider

- **Fast Track**
- **Priority Review**
- **Accelerated Approval**
- **Breakthrough Therapy**

Examples

Safety Assessment Studies for r-Human Insulin

- Extensive Chemical analysis
- Genetox (Ames, DNA repair, and SCE)
- Pyrogenicity
- Antigenicity (rats and guinea pigs)
- Acute toxicity (rats, mice, and dogs)
- One month studies (rats and dogs)

Hayes: Principles and Methods of Toxicology 2001

Safety Assessment for Neupogen® (G-CSF)

- Single dose studies in mouse, rat, hamster, monkey and juvenile rat – several routes
- 4-week studies in rat and monkey
- 13-week rat and monkey
- 52-week rat and monkey
- Full reproductive tox in rats and rabbits
- Antigenicity in rabbits, guinea pigs and mice
- General safety pharmacology studies
- Ames, chromosomal aberrations and mouse micronucleus

Modified Proteins

- Pegylation: eg. Neulasta™
- Fc Fusions: eg. Enbrel→
- Amino Acid changes:
  eg. (Aranesp ™)
- Conjugated proteins or antibodies (toxins or drugs)

Pegfilgrastim Toxicology Program

- Acute SC tox in rats
- 2-week SC tox in rats
- 3/6-month SC/IV tox in rats
- 1-month SC tox in cynomolgus monkeys
- Fertility SC in rats
- Embryo-fetal development SC in pregnant rabbits
- Embryo-fetal development SC in pregnant rats*
- Pre- and postnatal development SC in rats

*placental transfer, TK in pregnant rats conducted as separate PK study

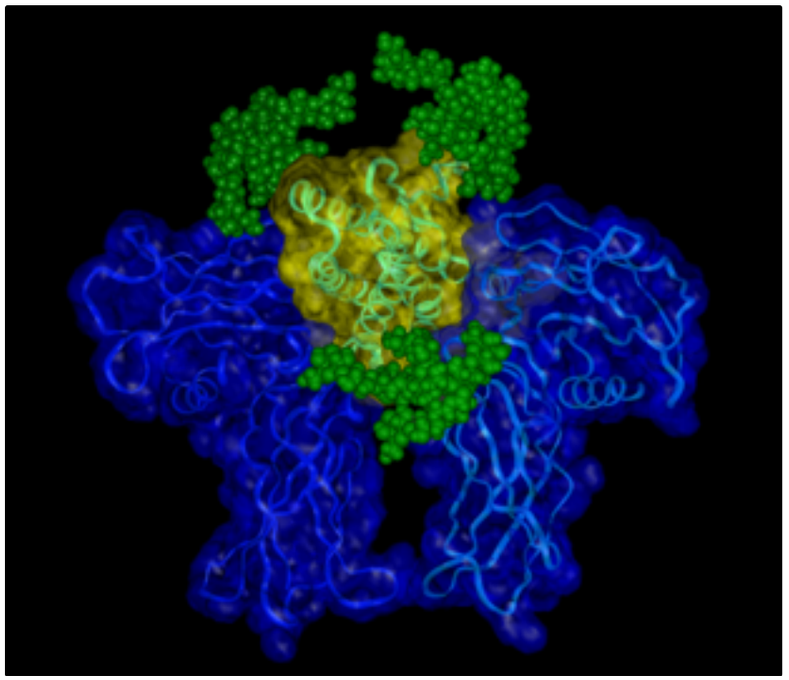
Through BLA/MAA filing

ENBREL→ (etanercept)

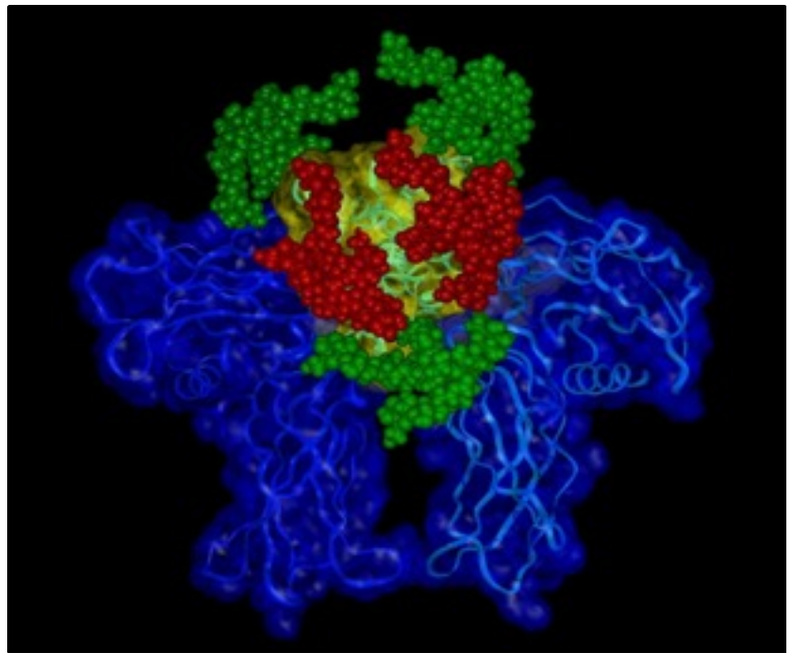
- 4-week Combo with an IL-1 inhibitor in monkeys, with 4-weeks recovery
- Mouse Host Resistance studies (KO mice)
- Immunotox in KO Mice
- Acute Monkey
- 2-week and 4-week Monkey
- 12-week Rat and Mice
- 6-month Monkey with Recovery
- Seg II Rabbit
- Seg I/II Rat
- Full Mutagenicity Package

Molecular Structure

rHuEPO



ARANESP™



ARANESP™ (darbepoetin alfa) has:

- **5 different amino acids in the protein sequence**
- **Two additional sialic-acid–containing carbohydrates (red)**
- **Up to 8 additional sialic acids**
- **Increased molecular weight (~37,100 daltons)**

Aranesp™ Toxicology Package

- Single dose Rat and Dog
- 1,3, and 6-month Rat and Dog with recovery
- Full genetox package (would not be performed now)
- Safety Pharmacology
- Full Reproductive Toxicology package
- Receptor binding study

Example: Phase 1 Enabling Toxicology Package for an Orally-Administered Small Molecule

- ✓ **Single dose escalation and 1-month continuous dosing phase 1**
- ✓ **Daily oral administration**
- ✓ **Healthy volunteers; males and non-reproductive females**

- ✓ Safety pharmacology
  - In vitro hERG current inhibition assay
  - Single dose CNS safety study in rats
  - Single dose CV/respiratory safety study in dogs
- ✓ Repeated-dose toxicity
  - 28-day oral toxicity study in rats
  - 28-day oral toxicity study in dogs
- ✓ Genetic toxicity
  - Bacterial mutagenicity (Ames)
  - In vitro chromosome aberration assay
  - In vivo mouse bone marrow micronucleus assay

Example: Phase 1-Enabling Toxicology Package for a Monoclonal Antibody

- ✓ **1-month MD phase 1, once-weekly IV dosing**
- ✓ **Trial in post-menopausal women with rheumatoid arthritis**
- ✓ **Molecule active in humans and monkeys**
- ✓ **Target not expressed in brain, heart, or lungs**

- ✓ Tissue Cross Reactivity Study (for MAb)
  - Tissues from human, monkey, rat, and rabbit
- ✓ Repeated dose toxicity study
  - 1-month toxicity study in monkeys (with safety pharmacology endpoints) with recovery

Example: Registration-Enabling Toxicology Program for Small Molecule

- Safety pharmacology
  - In vitro hERG current assay
  - CNS safety in rats
  - CV/respiratory safety in dogs
- Single dose toxicity
  - Rats and dogs
- Repeated dose toxicity
  - 1-month rat, 3-month rat, 6-month rat
  - 1-month dog, 3-month dog, 9-month dog
- Genotoxicity
  - In vitro Ames assay, in vitro chromosome aberration assay, in vivo mouse micronucleus assay
- Carcinogenicity
  - 2-year bioassay in rats
  - 6-month (short-term) bioassay in transgenic mouse model
- Developmental and reproductive toxicity
  - Fertility/general reproduction (rats), embryo/fetal development (rats/rabbits), peri-/postnatal development (rats)

Example: Registration-Enabling Nonclinical Toxicology Package for a Recombinant Human Protein

- ✓ **Short-term clinical use, IV and SC administration**
- ✓ **Oncology supportive care; men and women undergoing aggressive treatment for cancer**
- ✓ **Molecule active in humans, rats, rabbits, and monkeys**

- ✓ Single dose (IV and SC, rats/monkeys)
- ✓ Repeat dose (IV/SC, rats/monkeys)
  - Up to 28 consecutive days
- ✓ Safety pharmacology (IV, mice/rats/monkeys/Guinea pigs)
- ✓ *Genotoxicity (Program initiated before ICH S6)*
  - *Ames, chrom ab, mouse micronucleus*
- ✓ Reproductive toxicity (IV, rats and/or rabbits)
  - Segment 1 and Segment 2
- ✓ Local tolerance in rabbits (IV/SC/IM)
- ✓ In vitro hemolysis and antigenicity (mice/rats/Guinea pigs)

Example: Registration-Enabling Toxicology Program for Monoclonal Antibody

- ✓ **Cross-reacts with and functions in cynomolgus monkey (no others)**
- ✓ **Non-life threatening indication that requires chronic dosing**
- ✓ **Adult population of childbearing potential**

- ✓ Safety pharmacology
  - Single dose cardiovascular/respiratory/CNS safety in cynos
- ✓ Repeated dose toxicity
  - 1-month cyno, 6-month cyno
- ✓ Developmental and reproductive toxicity
  - Reproductive parameters evaluated in repeated dose studies, embryo/fetal development in cynos, pre-/postnatal development in cynos
- ✓ Special toxicity studies
  - Tissue cross-reactivity study with human and cyno tissues

Example: Surrogate Utilization for Efalizumab (Raptiva®)

- **Humanized IgG1 mAb to human CD11a**
- **Indication: moderate-severe plaque psoriasis**
- **Chronic administration -- Patient population men & women of childbearing age**
- ***Cross-reacted with only chimpanzee CD11a***

- 6-month tox study conducted in chimpanzees
  - *No histopathology* (non-terminal study)

- *Reproductive toxicology studies required due to patient population*
- Developed surrogate antibody
  - Chimeric rat/mouse anti-mouse CD11a antibody, called muM17

Example: Toxicology Program with Mouse Surrogate for Efalizumab

- Repeated-dose toxicity in mice (up to 6 months duration)
  - Terminal, with histopathology
- Reproductive toxicity in mice
  - Fertility/general reproductive toxicity
  - Embryo/fetal developmental toxicity
  - Peri-/Postnatal developmental toxicity
- Immunotoxicology
  - Adult mice
  - Offspring born to dams administered muM17 during gestation and lactation
- Others

Advice for FDA Meetings on Biologics

- **Biologics approaches "case-by-case"**
  - Regulatory guidance may not apply, out-of-date, open to interpretation
  - Biosimilars will require ↑ in FDA interactions

- **Gain upfront agreements**
  - Novel MOA/target
  - Novel development situations
  - Unexpected pharmacologic/toxicologic signals
  - Adequacy of data - US expectations

- **Deliberate input**
  - Agreements in writing
  - Continuity of advice
  - Reduces regulatory uncertainty

- **Use all "end of phase" (EOP) meetings**[20]
  - More difficult to get meetings
  - Generally 1 per phase

[20]Guidance for Industry – Formal Meetings Between the FDA and Sponsors or Applicants: US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Rockville, MD, USA . May 2009.

Summary

Biologics: Environment is challenging and changing

- Guidance documents, precedence and regulatory intelligence → a starting point
- Develop regulatory approaches based on unique biology of product
- Develop key messages
  - Be consistent across documentation types
- Form working partnerships with FDA early
  - Develop case-by-case solutions
  - Biosimilars will require even closer partnering with FDA
- Maintain consistent, ongoing and transparent communications (meetings, submissions, email)

Selected References

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