



Demonstrating Therapeutic Equivalence of Biotech-derived Products: Correlation of Structure-Activity Relationships (SAR) to Clinical Safety & Efficacy

- Therapeutic Equivalence is comprised of:
 - Analytical Comparability
 - Bioequivalence and Bioassay Comparability
 - Clinical Equivalence/ Non-Inferiority Trials
- Overview of SAR for Varying Classes of Products
- Correlation of SAR with Safety, Efficacy: Predictive Value
- Surrogate Endpoints: Relevance to Therapeutic Equivalence
- Utility of Clinical Bridging Studies vs. Post-Marketing Surveillance Studies

Demonstrating product comparability following process changes is predicated on knowledge of the relationship of product characteristics to safety, purity, and potency. Thus, it's not so much what the changes are as much as what they mean with regards to clinical safety and efficacy. Innovator firms stipulate the comparability exercise is only applicable to a single manufacturer with intimate knowledge of process, product, and methods. Innovator firms maintain that biotech manufacturing is too complex to be easily transferred - even within the same company - and thus, multisource firms must complete an entire safety and efficacy profile of their compounds; there are no innovator data comparisons that would enable a fast-track approach without compromising safety and efficacy. The competitor firms contend that the differences in process and analytical characterization from one manufacturer to another may be assessed using a similar approach, but with expanded characterization testing. Using a tiered testing approach based on product complexity, analytical methods sensitivity, and historical knowledge of clinical safety and efficacy profiles, a multisource approach is feasible. A central tenet of any comparability exercise is structure-activity-relationships (SAR). While innovator firms claim SAR is only applicable within a single manufacturer, multiple products (e.g., growth hormone, insulin, and interferon) suggest otherwise - that highly conserved features across all products can be assessed into decision trees and/or compendial monograph supplements.

A detailed discussion of the multisource biotech issues is provided in Attachments 1 through 4:

- Attachment 1: Regulatory and Development Issues in the Demonstration of Therapeutic Equivalence for Multisource Biotech-derived Pharmaceuticals, *Drug Information Journal* Vol 34(3) August 2000
- Attachment 2: Countdown to Biogenerics, *BioCentury* Vol 10(17), April 15, 2001
- Attachment 3: Biogeneric Science, *BioCentury* Vol 10(35), August 12, 2002
- Attachment 4: CPMP 3097/02 - Note for Guidance on Comparability of Medicinal Products Containing Biotechnology-derived Proteins as Drug Substance: Annex on Non-Clinical and Clinical Considerations, July 2002
- Attachment 5: Biologics, Biotechnology, Comparability, and Generics - Back to the Past, Curtis Scribner, MD, *FDLI Update* (2) 2001



Therapeutic Equivalence: Analytical, Bioequivalence, and Surrogate Endpoint Equivalence

- Analytical Comparability
 - Physico-chemical Comparisons
 - Confirmation of Primary, Secondary, and Tertiary Structures
 - Analysis of Differences: Purity vs. Impurities
 - Links with Manufacturing and Stability
 - Links to Bioassay and Surrogate Endpoints
 - Compendial reference standards: Cross-over studies
- Bioequivalence & Bioassay Comparability
 - Bioassay Comparability
 - Bioequivalence
- Clinical Equivalence/ Non-Inferiority Trials
 - Use of Validated Surrogate Endpoints
 - Establishing New Surrogate Endpoints

This is the holy grail. Analytical equivalence may need to be shown with multiple layers of testing - analogous to comparability testing currently done for innovator biotech products. Key aspects are safety, purity, and strength. Identity confirmed through MS-NMR, circular dichroism (CD), MALDI-TOF, SDS-PAGE, immunological profiles. Impurities confirmed through chromatographic methods, SDS-PAGE, TLC, but are not necessarily limited to the following: amino acid analysis; amino acid sequencing (entire sequence or amino- and carboxy-terminal sequences); peptide mapping; determination of disulfide linkage; SDS-PAGE (reduced and non-reduced); isoelectric focusing, HPLC (e.g., RP, SEC, ion-exchange, etc.); mass spectroscopy, assays to detect product-related proteins including deamidated, oxidized, cleaved, and aggregated forms and other variants (e.g., amino acid substitutions, adducts/derivatives); assays to detect residual host proteins, DNA, reagents; immunochemical analyses; assays to quantitate bioburden, endotoxin. Be intimately familiar with analytical method applications and limitations. Search the WEB for innovative technologies to apply to your product. Links to SAR discussed in later overheads.

Additional physicochemical characterization may be required for products undergoing post-translational modifications, for example, glycosylation, sulfation, phosphorylation, or formylation. Additional physicochemical characterization may also be required for products derivatized with other agents, including other proteins, toxins, drugs, radionuclides, or chemicals. The information submitted should include the degree of derivatization or conjugation, the amount of unmodified product, removal of free materials (e.g., toxins, radionuclides, linkers, etc.), and the stability of the modified product.

Bioequivalence for most drug products given via parenteral, otic, or ophthalmic routes are made with identical formulations to innovator and usually waive BE requirements. With biologics, bioassay comparisons may substitute partially (e.g., growth hormone, insulin), but with more complex products (e.g., interferon, interleukins, etc.) or life-threatening conditions (AMI, stroke, cancer) there may be assessment of PK to PD relationship, immunogenicity, and some link to impact on clinical markers associated with positive endpoints. Thus, the BE study for a biologics may encompass significantly more subjects, longer assessment periods, and safety/ immunogenicity testing vs. routine BE studies for drugs. Key aspect in design is knowing (1) the PK to PD relationship [PK<PD, PK=PD, or PK>PD] and (2) if innovator product is associated with known immunogenicity aspects (e.g., clotting factors [yes], immune globulins [no], growth hormone [yes, but very rare with any clinical impact], etc.).

See Attachment 6 - USP Chapters <1041> Biologics and <1045> Biotechnology-derived Articles for an overview of development stages and testing associated with various manufacturing schemes and product categories.



Overview of SAR for Varying Classes of Products

- **fibrinolytics** (e.g., Activase, Abbokinase)
- **human insulin** (e.g., Humulin)
- **monoclonal antibodies** (e.g., ReoPro, Herceptin, Oncoscint)
- **polyclonal antibodies** (e.g., CytoGam)
- **interferon** (e.g., Intron A, Roferon A, Alferon N)
- **interleukins** (e.g., Proleukin)
- **vaccines; monovalent vs. polyvalent; unconjugated vs. conjugated; adjuvant vs. none** (e.g., Engerix B, DPT, polio, measles, chicken pox, mumps, etc.)
- **somatotropins** (e.g., Protropin, Nutropin)
- **glucocerebrosidase** (e.g., Ceredase)

The Human Genome Project has given rise to the science of proteomics and the ~30,000 genes identified translate into 300,000 to 1 million proteins when alternate splicing and post-translational modifications are considered. While a genome remains unchanged to a large extent, the proteins in any particular cell change dramatically as genes are turned on and off in response to its environment. Thus, a reflection of the dynamic proteome has also been coined "functional proteome" to describe all the proteins produced by a specific cell in a single time frame. With the advent of DNA microarray, it's possible to express and monitor thousands of genes simultaneously. Genetic information is static while the protein complement of a cell is dynamic; thus, the functional proteome is seen in the multitude of proteins providing structural and functional framework for cellular life. Although DNA/RNA is easier to work with, there are limitations to the information that can be derived from DNA/RNA analysis. DNA sequence analysis does not predict if a protein is in an active form. RNA quantitation does not always reflect corresponding protein levels. Multiple proteins can be obtained from each gene when post-translational modification and mRNA splicing are taken into account. DNA/RNA analysis cannot predict the amount of a gene product that is made, if and when a gene will be translated, the type and amount of post-translational modifications, or events involving multiple genes such as aging, stress responses, drug responses and pathological transformations. Genomics and proteomics are complementary fields, with proteomics extending functional analysis.

Primary structure of peptides and proteins is the linear sequence of amino acids that are bound together by peptide bonds. Change in a single amino acid in a critical area of the protein or peptide can alter biologic function, as is the case in sickle cell disease and many inherited metabolic disorders. Disulfide bonds between cysteine (sulfur containing amino acid) residues of the peptide chain stabilize the protein structure. The primary structure specifies the secondary, tertiary and quaternary structure of the peptide or protein.

Secondary structure of peptides and proteins may be regular structures like an alpha helix, pleated sheet that may repeat, or the chain may organize itself randomly. The individual characteristics of the amino acid functional groups and placement of disulfide bonds determine the secondary structure. Hydrogen bonding stabilizes the secondary structure.

An inherent problem with SAR studies is that genomic information does not predict post-translational modifications that most proteins undergo. After synthesis on ribosomes, proteins are cut to eliminate initiation, transit and signal sequences and simple chemical groups or complex molecules are attached. Post-translational modifications are numerous (more than 200 types have been documented), static and dynamic including phosphorylation, glycosylation and sulfation.

Tertiary structure of proteins and peptides is the overall 3-D conformation of the complete protein. Tertiary structure considers the steric relationship of amino acid residues that may be far removed from one another in the primary structure. Such 3-D structure is that which is most thermodynamically stable for a given environment and is often subject to change with subtle changes in environment. In vivo, folding of large multi-domain proteins occurs co-translationally and the maturation of proteins occurs in seconds or minutes. Intracellular protein folding is regulated by cellular factors to prevent improper aggregation and facilitate translocation across membranes. The two methods for determining 3-D protein structures are nuclear magnetic resonance and x-ray crystallography.

If the functional protein consists of several subunits, the quaternary structure consists of the conformation of the entire subunits bound together by electrostatic and hydrogen bonds. Multi-subunit proteins are called oligomers and the various component parts are each monomers or subunits. Proteins may contain non-amino acid functional structures such as a vitamin derivative, mineral, lipid or carbohydrate.



Fibrinolytics: Activase (alteplase)

- **Physico-chemical Description:** a serine protease (glycoprotein of 527 AA) which enhances the fibrin conversion of plasminogen to plasmin; see USP reference for compendial testing profile
- **Clinical Indications:** acute myocardial infarction (AMI), acute ischemic stroke, pulmonary embolism
- **Approved Products:** Activase (alteplase) (Genentech);
- **Method(s) of Manufacture:** complementary DNA (cDNA) from a human melanoma cell line introduced into a Chinese Hamster Ovary (CHO) cell line + gentamycin (selection pressure)
- **Analytical Testing Considerations:** biological potency assessed by in vitro clot lysis assay vs. WHO standard; alteplase activity = 580,000 IU/mg; bulk sterile material assayed for viral, mycoplasma, DNA contamination, and molecular identity (e.g., tryptic mapping, specific activity, & protein content); finished product tested for appearance, sterility, safety, pyrogenicity, identity, purity, potency, pH, inorganic phosphate content, arginine content, polysorbate content, moisture, and fill volume.

Alteplase:

Approved Nov 1987 under the Office of Biologics Research and Review for AMI; approved June 1990 for pulmonary emboli

Master Cell Bank testing for adventitious agents included: direct cultivation, inoculation of indicator cell cultures and animals, hemadsorption, hemagglutination, immunogenicity, enzyme assay, electron microscopy, radioimmunoassay, and radioisotopic labelling.

Manufacturing process validated for removal of DNA, foreign proteins, and culture medium additives, plus the inactivation and removal of model retroviral particles.

Small scale process during development and clinical trials used roller bottle fermentation that produced a two-chain protein linked by disulfide bond. Large scale commercial process used a suspension culture technology resulting in a one-chain form. In vitro and in vivo thrombolysis testing showed product comparable. PK data showed the clearance of roller-bottle material was slower than suspended culture material; a 100 mg dose of suspended culture material was equal to 80 mg of roller bottle material.

In theory, alteplase should be effective only at the surface of fibrin clot. In practice, however, a systemic lytic state is seen, with moderate amounts of circulating fibrin degradation products and a substantial systemic bleeding risk. The agent may be re-administered as necessary, as it is not antigenic and almost never is associated with any allergic manifestations.

Plasminogen activator inhibitor 1 (PAI-1), the main physiological inhibitor of tissue-type plasminogen activator (t-PA) can occur in three different conformations, each exhibiting a different function (i.e. latent, inhibitory or substrate). Mutagenesis, fluorescence spectroscopic analysis and conformation-specific and neutralizing monoclonal antibodies are used to study the structure-function relationship in the PAI-1 molecule. Detailed understanding of the biochemical and conformational regulation of PAI-1 activity may advance efforts to prevent and treat thrombotic disorders associated with increased PAI-1 levels.

See **Attachment 8** for USP monographs of alteplase and alteplase for injection.



Fibrinolytics: Reteplase (r-PA, Retevase)

- **Physico-chemical Description:** a synthetic non-glycosylated deletion mutation of t-PA containing 355 of the 527 amino acids comprising native t-PA
- **Clinical Indications:** acute myocardial infarction (AMI) with off-label uses in acute ischemic stroke, pulmonary embolism, and venous thrombosis
- **Approved Products:** Activase (alteplase) (Genentech);
- **Method(s) of Manufacture:** E. coli
- **Analytical Testing Considerations:** similar to alteplase - potency assessed by in vitro clot lysis assay vs. WHO standard; bulk sterile material assayed for viral, DNA contamination, and molecular identity (e.g., tryptic mapping, specific activity, & protein content); finished product tested for appearance, sterility, safety, pyrogenicity, identity, purity, potency, pH, inorganic phosphate content, arginine content, polysorbate content, moisture, and fill volume.

Reteplase:

Reteplase (r-PA, Retevase) is a second-generation recombinant tissue-type plasminogen activator that seems to work more quickly and to have a lower bleeding risk than the first-generation agent alteplase.

The drug is produced in *Escherichia coli* by recombinant techniques. Reteplase does not bind fibrin as tightly as native tissue plasminogen activator, allowing the drug to diffuse more freely through the clot rather than binding only to the surface the way tissue plasminogen activator does. In high concentrations, reteplase does not compete with plasminogen for fibrin-binding sites, allowing plasminogen at the site of the clot to be transformed into clot-dissolving plasmin. These 2 modifications help explain the faster clot resolution seen in patients receiving reteplase than in those receiving alteplase.

The modifications also resulted in a molecule with a faster plasma clearance and shorter half-life (about 11-19 min) than alteplase. Reteplase undergoes renal (and some hepatic) clearance. The shorter half-life makes the drug ideal for double-bolus dosing. The result is more convenient administration and faster thrombolysis with reteplase than with alteplase, which is given by a bolus followed by an intravenous (IV) infusion.

The agent may be readministered as necessary, as it is not antigenic and almost never is associated with any allergic manifestations.



Fibrinolytics: Abbokinase (urokinase)

- **Physico-chemical Description:** a serine protease (glycoprotein of 527 AA) which enhances the fibrin conversion of plasminogen to plasmin;
- **Clinical Indications:** acute myocardial infarction (AMI), pulmonary embolism, and IV catheter clearance
- **Approved Products:** Abbokinase (urokinase) (Abbott Labs);
- **Method(s) of Manufacture:** cultured from human kidney cells (removed from newborns who died)
- **Analytical Testing Considerations:** biological potency assessment - also by in vitro clot lysis assay vs. WHO standard? Bulk sterile material probably assayed for viral and mycoplasma contamination, molecular identity (e.g., tryptic mapping, specific activity, & protein content). Finished product tested probably tested for appearance, sterility, safety, pyrogenicity, identity, purity, potency, pH, moisture, and fill volume.

Abbokinase:

Approved by CBER in December 1983 under Section 505 of the FD&C Act

Method of manufacture (tissue culture technique from deceased newborns) came under fire from FDA in January 1999 with a "Dear Doctor" letter advising use should be "reserved only for those situation where a physician has considered the alternatives and has determined that the use of Abbokinase is critical." This warning/ admonition came after FDA inspectors halted shipments of Abbokinase in November 1998 following "significant deviations" from manufacturing safety and purity. Chiefly was the kidney cells removed from deceased foreign newborns that were at high risk of infectious disease - including hepatitis C, but Abbott's testing didn't account for this screening. While FDA notes Abbott's measures could have allowed contamination, there wasn't any finding of contamination. Shipments were allowed to resume in Jan 1999 with heightened manufacturing measures in place.

Master Cell Bank testing for adventitious agents most likely comparable to Genentech (and others) which included: direct cultivation, inoculation of indicator cell cultures and animals, hemadsorption, hemagglutination, immunogenicity, enzyme assay, electron microscopy, radioimmunoassay, and radioisotopic labelling.

Manufacturing process most likely validated similar to Genentech (and others) for removal of DNA, foreign proteins, and culture medium additives, plus the inactivation and removal of model retroviral particles.

Urokinase (Abbokinase) is the fibrinolytic agent most familiar to interventional radiologists and the one that has been used most often for peripheral intravascular thrombus. At the time of this writing, urokinase is not available from the manufacturer. Its availability in the immediate future is not known. In the meantime, the FDA has encouraged the off-label use of reteplase and alteplase for local-regional lysis of venous and arterial thrombus at any location. Urokinase is a physiologic thrombolytic agent that is produced in renal parenchymal cells. Unlike streptokinase, urokinase directly cleaves plasminogen to produce plasmin. When purified from human urine, approximately 1500 L of urine are needed to yield enough urokinase to treat a single patient. Urokinase is also commercially available in a form produced by tissue culture, and recombinant DNA techniques have been developed for urokinase production in E coli cultures.

In plasma, urokinase has a half-life of approximately 15 minutes. Allergic reactions are rare, and the agent can be administered repeatedly without antigenic problems.

See **Attachment 8** for article on SAR of urokinase inhibitors.



Human Insulin

- **Physico-chemical Description:** a protein (MW = 5807.69) with two chains; Chain A (21 AA) is linked to Chain B (30 AA) via cysteine linkages at positions # 7 and 20 (on Chain A) to positions # 7 and 19 (on Chain B). Insulin available in 6 formulations: **R** (regular), **N** (NPH), **L** (Lente), **U** (Ultralente), **50/50 NPH:R**, and **70/30 NPH:R**
- **Clinical Indications:** treatment of diabetes
- **Approved Products:** Humulin R (Lilly) and Humalog (lispro injection) (Lilly)
- **Method(s) of Manufacture:** rDNA expression in *E. coli* or by chemical modification of pork insulin; re-folding efficiencies only about 30% but still cheaper to do via *E. coli* than mammalian cell lines; arginine used a lot in formulations to protect protein
- **Analytical Testing Considerations:** see USP standards for Insulin, Insulin Injection, Insulin Human, Insulin Human Injection, Isophane Insulin Suspension, Insulin Zinc Suspension, Extended Insulin Zinc Suspension, and Prompt Insulin Zinc Suspension

Background: Insulin from bovine and porcine sources have been used for the last 50 years. Human insulin (Humulin) was approved in March 1994. Advantages over animal-derived insulin include a greater safety margin - no possible transmission of epizootic infections such as BSE, TSE, prions, or unknown organisms.

Insulin available in 6 formulations:

R (regular): zinc-insulin solution, nothing is added to alter speed or duration of activity, which is rapid onset and short duration (4-12 hours)

N (NPH): isophane suspension is insulin with protamine sulfate and zinc yielding an intermediate action with slower onset and longer duration of activity up to 24 hours

L (Lente): zinc-insulin suspension is an intermediate acting with slower onset and longer duration of activity up to 24 hours

U (Ultralente): extended zinc suspension yielding a long-lasting action with slower onset and longer duration of activity up to 28 hours

50/50 (NPH:R): is a formulation of 50% Human Insulin Isophane Suspension (NPH) with 50% Human Insulin Injection (buffered regular). This is more intermediate acting insulin combined with rapid onset insulin to give a combined activity up to 24 hours duration.

70/30 (NPH:R): is a formulation of 70% Human Insulin Isophane Suspension (NPH) with 30% Human Insulin Injection (buffered regular). This is more intermediate acting insulin combined with rapid onset insulin to give a combined activity up to 24 hours duration.

Humalog (insulin lispro injection) is a rDNA insulin analog with Lys (B28) and Prot (B29) that is faster acting than regular insulin. It's used to treat hyperglycemia and often used in combination with other insulin regimens.

See **Attachment 9** for compendial data and background on Humulin production methods, as well as noted differences in safety and efficacy vs. animal-derived insulin.



Monoclonal Antibodies

- **Physico-chemical Description:** Several classes with varying properties for each: IgA, IgG, IgM, IgE, etc., plus sub-types.
- **Clinical Indications:** colorectal cancer imaging (Oncoscint), treatment of certain patients with metastatic breast cancer (Herceptin), prevention of ischemic complications post-PTCA (ReoPro), non-Hodgkin's lymphoma (Rituxan)
- **Approved Products:** Herceptin (Genentech); Rituxan (Genentech); ReoPro (Centocor); Oncoscint (Cytogen)
- **Method(s) of Manufacture:** murine hybridoma cell lines; surface epitope masking (SEM) may be used to identify and develop certain Ab sub-types
- **Analytical Testing Considerations:** functional assays such as antigen-binding, neutralization assays, PK profiles; biochemical tests such as cyanogen bromide (CNBr) cleavage following N-terminal sequencing, peptide mapping, comparison of impurity & degradation profiles (via SDS PAGE), carbohydrate analysis, SEC, DSC, capillary electrophoresis, etc.

- Some safety/ clinical issues for monoclonal development include:
 - **Cross-reactivity** of the monoclonal with normal tissues: can lead to inappropriate targeting of other tissues with acute or chronic/ irreversible effects
 - **Toxicity due to the toxin conjugate or radioactive moiety:** monoclonals linked to radioisotopes or toxins that "unlink" can release toxic fragments to other organs
 - **Initiation of patient's immune response against the foreign antibody:** neo-antigens or neutralizing antigens can (1) negate the benefit of the monoclonal and (2) may combine to create a circulating immune complex (CIC), which upon deposition in certain organs (e.g., pulmonary) can exacerbate condition or create new life-threatening scenarios (e.g., serum sickness, allergic reactions). Most monoclonals are murine hybridomas and thus, may be recognized as foreign by host - commonly referred to as Human Anti-Mouse Antibodies (HAMA) - a well recognized effect by regulatory bodies. Clinical development should include PK data on formation and elimination of HAMAs. Some human chimeric monoclonals may produce monoclonals that do not produce HAMAs.
 - **Impurities which may contaminate or infect the patient:** may be impurities from cell lines so, methods of manufacturing must be validated for removal/ inactivation of adventitious agents.
- A number of regulatory guidance documents detail characterization testing and manufacturing considerations. As noted in comparability protocol guidance documents/ articles, be prepared to link extensive analytical testing to manufacturing process development and stability.

See **Attachment 10** for an article summarizing key development of monoclonal antibodies.



Polyclonal Antibodies

- **Physico-chemical Description:** gamma globulins
- **Clinical Indications:** adjunct to antibiotics in treatment or prevention of infectious diseases (e.g., respiratory syncytial virus [RSV], *P. aeruginosa* infection in cystic fibrosis patients, *S. aureus* infections in renal dialysis patients, treatment of ITP in AIDS patients, etc.)
- **Approved Products:** RespiGam & Cytogam (Medimmune), WinRho SD (Cangene), Gamimmune (Bayer), Sandoglobulin (Sandoz)
- **Method(s) of Manufacture:** gamma globulins isolated by fractionation from pooled source plasma donors (either vaccinated or naturally high-titered individuals). See GMP requirements for blood and blood component collection facilities (21 CFR 606 & 640(j)).
- **Analytical Testing Considerations:** From the early 30's to the 60's, source plasma had high Fc fragments that precluded IV use (thus limited to IM administration). In the '70's, improved manufacturing allowed low enough Fc fragment levels for IV Rx - now known as IGIV. Antibody characterization testing for activity/ specificity.

• Throughout the 70's and 80's, sloppy practices in blood collection facilities (e.g., poor screening/ rejection of ineligible/ infected donors, poor/ no tracking of infected units, no look-back regulations, and inconsistent "manufacturing practices"). These collection and processing pitfalls - when combined with source plasma fractionating procedures that didn't include viral inactivation/ removal validation - created one of the largest iatrogenic epidemics of AIDS, HTLV, and hepatitis among blood recipients ever known. In the late '80's, the Red Cross entered into a consent decree with FDA to implement GMPs for blood collection and processing centers. In combination with better tracking and diagnostic kits, the blood supply has restored confidence.

• Source plasma fractionation may be accomplished two major ways: cryoprecipitation or chromatographic separation. Cryoprecipitation allows fractionation of albumin and coagulation factors from gamma globulins. Chromatographic separation may be used for certain sub-portions of isolation. All processes today routinely use viral inactivation/ removal methods to ensure contaminated units (that slip by testing) do not compromise patient safety. Albumin is usually pasteurized via high heat. Solvent/ detergent methods with Triton X/ TNP are useful for inactivating lipid enveloped viruses. Methods also need to demonstrate removal of solvent/ detergent components.

• Some polyclonals were developed by identifying donors with naturally high titers (e.g., nurses with exposure to RSV had high anti-RSV titers and Rh- women (post-partum to Rh+ babies) had high anti-Rh+ antibodies). Indeed, one Rh- woman had such extraordinarily high titers that the company using her plasma paid for her complete relocation to Florida (from Canada) just to continue using her plasma.

• Titers (to highly specific antigens) may be cultivated via source plasma donor screening followed by proprietary vaccination programs. Existing source plasma donors are vaccinated with proprietary vaccines and the high-titered individuals/ units are segregated for special production - resulting in "high octane" gamma globulins. This technique has been used in development of polyclonals to *P. aeruginosa* (for Rx in cystic fibrosis), *S. aureus* Type 5 and 8 bivalent (for Rx in renal dialysis patients), gp120 (for Rx in needle-stick injuries from HIV+ individuals), etc. Some newer technologies are allowing for expression of human polyclonals in some animal chimerics - thus avoiding the source plasma fractionation route. Another disadvantage of the source plasma route is that donor supplies are dwindling with increased regulatory/ testing scrutiny.

See **Attachment 10** for an overview of glycosylation relationships to binding and functional activation. See **Attachment 11** for summary minutes of the May 2002 PPTA-FDA Workshop on the Comparability and Bioequivalence of Plasma Derivatives in the Fractionation Industry.



Interferon (cytokines)

- **Physico-chemical Description:** Five major classes - 2b-alpha is the most pleotropic with immunomodulatory, anti-proliferative, antiviral, and antimicrobial activity. Actions include enhancement of phagocytosis to augmented cytotoxicity. See notes below for specifics of each interferon type
- **Clinical Indications:** multiple sclerosis, chronic granulomatous disease, hairy cell leukemia and AIDS-related Kaposi's sarcoma, genital warts, hepatitis, etc.
- **Approved Products:** Alferon N (Purdue Frederick); Intron A (pegylated and non-)(Schering Plough); Roferon A (Roche); Actimmune A (Genentech); and Betaseron A (Chiron/ Berlex)
- **Method(s) of Manufacture:** rDNA in E. coli or CHO cells or induction from human leukocytes following incubation with viral challenges; purification with immunoaffinity chromatography, acidification, and gel filtration chromatography.
- **Analytical Testing Considerations:** See details below.

•Alpha n3 (human leukocyte derived); **Alferon N Injection:** 166 AA; molecular weight ranges 16,000 - 27,000 Da; manufactured from pooled units of human leukocytes induced by infection with avian virus (Sendai virus). Used to treat genital warts.

•Alpha 2b (rDNA derived from E. coli [vector from human leukocytes]); **Intron A:** molecular weight 19,300 Da. Used to treat hairy cell leukemia, genital warts, AIDS-related Kaposi's Syndrome, chronic hepatitis Non-A, Non-B/C (NANB/C), hepatitis B.

•Alpha 2a (rDNA derived from E. coli [vector from human leukocytes]); **Roferon A:** 165 AA; molecular weight about 19,000 Da; purified with immunoaffinity chromatography via a murine MAb. Used to treat hairy cell leukemia; AIDS-related Kaposi's Sarcoma

•Gamma 1b (rDNA derived from E. coli [vector from human leukocytes]); **Actimmune A:** 140 AA; molecular weight about 16,500 Da; active is a non-covalently bound dimer of two monomers. Used to treat Chronic Granulomatous Disease.

•Beta 1b (rDNA in CHO cell line adapted for suspension culture); 166 AA; molecular weight about 22,500 Da. **Betaseron A or Avonex.** Used to treat multiple sclerosis.

•NOTE: Pivotal clinical trials were conducted with material from an adherent cell culture (BG9015) but commercial production was from a suspended cell culture (BG9418). Comparability studies included: anti-viral and anti-proliferation activity of MHC class I expression; peptide maps, N-terminal sequencing, carbohydrate analysis, immunoblotting (e.g., IEF) analysis, RP-HPLC, receptor binding and other functional assays, and PK studies in humans; conclusion was the two cell lines produced comparable material.

•Stability studies include: **BULK MATERIAL** - appearance, protein concentration, potency/ specific activity/ SDS PAGE (reduced), size exclusion chromatography (for aggregates), peptide map, particulates (HIAC), and endotoxin (LAL). **FINISHED PRODUCT** - appearance, pH, content by ELISA, potency (via anti-viral assay), residual moisture, particulates (HIAC), sterility, and endotoxin (LAL).

•See discussion of interferon approval and SAR in **Attachment 12.**



Interleukins

- **Physico-chemical Description:** lymphokines with multiple effects on cell-mediated and humoral immune responses; IL-2 molecular weight about 15,300 Da.
- **Clinical Indications:** adjuncts to chemotherapy (e.g., metastatic renal carcinoma), AIDS, off-label uses, etc.
- **Approved Products:** Proleukin (aldesleukin) (Chiron Therapeutics); IL-4 and IL-10 under clinical development by several firms
- **Method(s) of Manufacture:** (for Proleukin) rDNA production in *E. coli* resulting in a non-glycosylated version of IL-2; rDNA in CHO or suspended tissue cultures
- **Analytical Testing Considerations:** Assess biological potency against external reference standard (e.g., native human or WHO reference).

No formal FDA guidance on manufacturing or testing requirements, but should apply a number of same testing requirements as for master cell banks, tissue culture qualification, etc.

See **Attachment 13** for an overview of EC registration of Proleukin via the Concertation Procedure.



Vaccines

- **Physico-chemical Description:** monovalent vs polyvalent; conjugated vs. not; formulated with adjuvants; etc.
- **Clinical Indications:** prevention of infectious disease (e.g., small pox, measles, polio, DPT, etc.); boosting titers as part of therapy (e.g., adjunct to cancer Rx); boosting antibody titers in source plasma donors for creation/ collection of specified high-titer antibodies (e.g., polyclonal Ab collection in passive immunotherapy)
- **Approved Products:** See PDR for complete listing
- **Method(s) of Manufacture:** microbial fermentation, tissue culture, egg culture, animal colonies. See May 1998 FDA guidance for details on vaccine CMC documentation.
- **Analytical Testing Considerations:** See May 1998 FDA guidance; testing varies according to vaccine type and manufacturing considerations (see below). See 21 CFR 610.53 for stability expiry dating periods. See 21 CFR 620 for additional testing for bacterial vaccines (e.g., pertussis, typhoid, anthrax, cholera, & BCG). See 21 CFR 630 for additional standards for vial vaccines.

Analytical testing varies considerably for each type of vaccine. Monovalent/ polyvalent antigens derived from straight fermentation require characterization testing for purity, immunospecificity, etc. Recombinant cell lines in either microbial, yeast, tissue culture, or avian egg lines require characterization testing of vector, host systems, and combined expression construct - as well as resulting bulk antigen. For conjugated antigens, additional testing of reaction completion (for conjugation steps) may be required with IPC and finished product/ stability testing to evaluate unconjugated antigen in container/ closure system. For polyvalent antigens, may be additional testing to ensure proper proportions in finished dosage form and consistent antibody responses in animal screening models.

For clinical demonstration, surrogate endpoints of GMT and seroconversion may be useful in predicting protective titers (e.g., > 10,000 GMT for hepatitis B vaccine).



Somatotropins

- **Physico-chemical Description:** rDNA polypeptide of 191 amino acids (Humatrope); 192 AA (Protropin) - the extra AA being methionine; both with a molecular weight about 22,000 Da
- **Clinical Indications:** long-term treatment of children with growth failure (Humatrope); AIDS-related complex; chronic renal insufficiency
- **Approved Products:** Humatrope (Lilly); Nutropin or Protropin (Genentech); Bio-tropin (Bio-Technology General Corporation); Genotropin (Pharmacia); Nordiotropin (Novo Nordisk); Saizen (Serono Laboratories)
- **Method(s) of Manufacture:** rDNA in *E. coli* (Humatrope),
- **Analytical Testing Considerations:** bioactivity assessed in hypophysectomized rat model against WHO standard (3 IU)/ mg); neo-antigen testing; peptide maps, FAB MS, RP-HPLC, Raman and CD spectra, electrophoresis, tryptic mapping, and bioassay

Background: Pituitary-derived hGH was used for treatment of short children for about 30 years, but the material was derived from collection & processing of cadaver pituitary glands - a practice now known to be inherently unsafe. During the review of Genentech's met-hGH, there were 3 documented cases of Creutzfeldt-Jakob Disease (CJD) from infected pituitaries. All the manufacturers of pituitary-derived hGH removed their products from the market.

Protropin (somatrem) (met-hGH [Genentech]) was approved after 3 years in clinical trials with up to 86 patients (treated up to 3 years). The major issue with met-hGH was antigenicity; about 42% of patients developed anti-hGH antibodies. One patient developed such strong antibodies that growth was arrested, however, upon cessation and resumption of treatment, the growth continued.

Regulatory Approval History:

Protropin (Genentech) approved Oct 1985; Humatrope (Lilly) approved March 1987; Nutropin (Genentech) approved Nov 1993; Bio-tropin (Bio-Technology General Corporation) approved May 1995; Genotropin (Pharmacia) approved Aug 1995; Nordiotropin (Novo Nordisk) approved May 1995

Orphan Drug Designations (ODD): Protropin and Nutropin (Genentech) for (1) long-term treatment of children who have growth failure due to a lack of adequate endogenous growth hormone secretion and (2) treatment of short stature associated with Turner's syndrome. Saizen (Serono Labs) for (1) treatment of idiopathic or organic growth hormone deficiency in children with growth failure, (2) enhancement of nitrogen retention in hospitalized patients suffering from severe burns, and (3) treatment of AIDS-associated catabolism/ weight loss. Humatrope (Lilly) for (1) treatment of short stature associated with Turner's syndrome and (2) long-term treatment of children who have growth failure due to inadequate secretion of normal endogenous growth hormone. Biotropin (BTG Corp) for treatment of cachexia associated with AIDS. Somatropin (Pharmacia) for treatment of adults with growth hormone deficiency. Norditropin (Novo Nordisk) for treatment of growth failure in children due to inadequate growth hormone secretion.

Humatrope (Lilly) application was reviewed and approved under Division of Metabolism and Endocrine Drug Products, then under Office of Biologics Research and Review.

FDA intends to issue guidelines on demonstrating therapeutic equivalence for growth hormone in 2002. These guidelines ... in conjunction with the guidance on uses of 505(b)(2) applications - or Paper NDAs - will allow multisource manufacturers to register rDNA growth hormone products with expedited development.



Glucocerebrosidase

- **Physico-chemical Description:** naturally occurring *GCR* is 497 amino acids and approximately 12% carbohydrate; molecular weight about 67 kDa. The modified version - *Ceredase* (alglucerase) is also 497 amino acids but only 6% carbohydrate; MW = 59.3 kDa.
- **Clinical Indications:** treatment of *Gaucher's disease*, a rare genetic disorder with a functional deficiency in the enzyme glucocerebrosidase (*GCR*) that results in the accumulation of lipids in tissue macrophage cells
- **Approved Products:** *Ceredase* and *Cerezyme* (*Genzyme*)
- **Method(s) of Manufacture:** rDNA in *E. coli* (*Humatrope*),
- **Analytical Testing Considerations:**

Background: *Ceredase* compared to naturally-derived *GCR* shows that *Ceredase* oligosaccharide chains were modified, altering the sugar residues at the non-reducing ends of the glycoprotein so that they predominately terminate with mannose residues, which are recognized by the carbohydrate receptors on the macrophage cell.

Sponsor claims the two have demonstrably different clinical profiles, but the naturally occurring *GCR* was tested in a single patient during a NIH trial (with modest effect) and compared to the controlled trial of 13 patients. Sponsor claims the clinical data was so compelling, FDA approved NDA on the basis on the 13-patient study.

Regulatory Approval History:

Ceredase (alglucerase) (*Genzyme*) approved April 1991; *Cerezyme* (imiglucerase) (*Genzyme*) approved 1994; Orphan Drug Designations (ODD): for treatment of *Gaucher's disease*

Alglucerase (*Ceredase*®) and imiglucerase (*Cerezyme*®) are approved for enzyme replacement therapy in Enzyme Replacement Therapy (ERT).

Initial doses of 30-60 U/kg of body weight every 2 weeks are considered safe and effective. Rapid improvements in hepatosplenomegaly, anemia, thrombocytopenia and quality of life have been demonstrated.(8,11-13) Doses as low as 15 U/kg administered every 2 weeks or 2.3 U/kg three times weekly have shown improvement in some hematological and visceral parameters.(10,14).

Dosing of *Cerezyme*® or *Ceredase*® is individualized for each patient based on disease severity and rate of progression as assessed by clinical, laboratory, and radiological evaluation. The U.S. Regional Coordinators of the International Collaborative *Gaucher* Group (ICGG), a panel of independent physicians who have extensive experience in the care of *Gaucher* patients, have made recommendations for dosing based on risk assessment for irreversible morbid complications.(1) These recommendations are summarized in Appendix 1 and 2.

Dosing adjustments after initiation of ERT are individualized, based on achievement of therapeutic goals as assessed by monitoring of each patient's progress. Recommendations for monitoring patients on ERT include hematological indices (such as hemoglobin, acid phosphatase, and AST/ALT levels), spleen and liver volume, DEXA scan for bone density, and quality of life questionnaire. Data suggest that different organs respond differently to ERT. While hematologic and visceral parameters usually improve within the first year, the response of the skeletal system is slower to occur and more difficult to measure. Evidence of skeletal improvement may require up to 2-3 years of therapy and may require doses of ERT that are greater than that which is necessary to observe improvement in hematological or organ parameters.(11,17) Failure of a patient to respond to therapy in 6 months typically indicates that a higher dose may be necessary.

ERT is lifelong treatment. Prolonged periods off therapy are not appropriate. Interruption in therapy is associated with relapse in hematological and organ volume parameters and is presumed to also result in relapse of skeletal parameters.



Surrogate Endpoints: Relevance to Therapeutic Equivalence

- **Vaccines:** Geometric Mean Titer (GMT) and seroconversion (vs. demonstrated prophylaxis from long-term studies)
- **Fibrinolytics:** in vitro clot lysis, post-MI (myocardial infarction) patency, ventricular function testing (ECG), etc. (vs. survival rates)
- **Somatotropins:** hypohysectomized rat model and comparison to external reference standards (vs. long-term growth confirmation)
- **Cystic Fibrosis Rx:** pulmonary function testing (PFT), days in ICU, days on intravenous antibiotics (vs. reduced number of exacerbations and long-term survival)
- **HIV/ AIDS:** reduction in CD4 count, viral burden, reduced rate of opportunistic infections (vs. survival rates)
- **Arthritis:** radiological imaging of joint damage, reduction of inflammatory mediators, improved joint mobility, etc.
- **Chemotherapeutics:** immunological mediators, carcinogenic antigen levels, lymph node involvement (vs. long-term survival)
- **Hepatitis C:** disappearance of Hepatitis C Virus RNA within 2 days is predictive for response to high-dose IFN 2b-alpha in chronic hepatitis C

Surrogate endpoints are valuable determinants when primary efficacy endpoints of morbidity or mortality (1) take too long to assess in chronic disorders, or (2) may become unethical delays in life-threatening conditions. Surrogate endpoint determinations/ equivalence is a moving target ... an evolving science. New diagnostic techniques and correlations are coming up all the time. One needs to assess the clinical condition with the most current methods available. Surrogate endpoints used 20 years ago are likely to be outdated; even some a few years old will be dated - especially when dealing with life-threatening conditions like burns, smoke inhalation, trauma, AIDS, cancer, sepsis, ALS, and others.

Also note that the innovator manufacturing may have changed substantially since approval and the clinical endpoints outlined in a SBA or referenced articles may not reflect the most current product. You will need to research this area thoroughly and be able to explain/ defend the scientific relevance (or not) of any observed microheterogeneity in your product vs. surrogate endpoint analysis. There is already precedence for therapeutic equivalence of two different manufacturers of menotropins, despite the microheterogeneity of the two materials. Thus, there can be analytical or physico-chemical differences with little or no clinical impact - but it's up to the sponsor to show this.

Remember that you cannot rely on an innovator's clinical efficacy data to support your application; yours must stand alone. Unless the innovator compound is approved under Section 505 of the FD&C Act and subject to a 505(j) or 505(b)(2) application, there is no generic biologic mechanism available. All applications must contain sufficient preclinical, clinical, and CMC data to stand alone. Thus, you need to make the best scientific case for analytical equivalence + bioequivalence + surrogate endpoint equivalence of your compound versus an innovator compound or external reference standard.

Development of fast-track surrogate endpoints facilitated quicker reviews. Will need to do extensive research of the innovator application, reviewing division thoughts, and clinical practice to determine the most clinically relevant and achievable surrogate endpoints. If no surrogate endpoints were done with innovator, may need to establish them. As with mathematical equation, If $A = B$ and $B = C$, then $A = C$. Development of surrogate endpoints may be achieved through limited clinical trials comparing innovator to generic for key clinical parameters. Examples of surrogate endpoints that have been developed for various conditions include:

Alteplase in coronary infarction, primary endpoints was global ventricular function (patency assessed by radioventriculography) but additional endpoints were artery patency and clinical outcome (death, congestive heart failure, and recurrent ischemia) (274 patients)

pulmonary emboli, primary endpoint was embololysis assessed by repeat pulmonary angiography at two hours; secondary endpoints included change in pulmonary hemodynamics at two hours and lung perfusion as assessed by radionuclear methods at 24 hours (45 patients)

cystic fibrosis therapy, primary endpoints were pulmonary function testing, days in ICU, days on IV antibiotic; secondary endpoints include quality of life,

What if your product doesn't match innovator in surrogate endpoints? You need to establish noise-to-signal ratio for the clinical benefit and see where your product fits in. Also consider doing multiple surrogate endpoints to assess a lot of clinical parameters (e.g., for fibrinolytics look at patency, cardiac output, diagnostic imaging, days in ICU, quality of life assessments, and other aspects). See **Attachment 14** for overview of surrogate endpoint analysis and non-inferiority trial design.



Immunogenicity

- **Plasma Derivatives:** IGIV generally not immunogenic but clotting factors (Factor VIII) and thrombopoietin (TPO) are. Epitope mapping may be useful in predicting antigenic regions.
- **Ceredase:** About 13% (of 509 patients);
 - average time to develop Abs was about 5 months but 90% of all patients who developed Abs did so within 9 months.
 - Majority of patients showed decreasing Abs over time.
 - 25% of patients who developed Abs had allergic symptoms, but only half of those patients had detectable Abs.
- **Monoclonal Antibodies (MAbs):** MAbs are inherently antigenic; assessed for each product.
 - Fab and Fab' fragments with 1-8%.
 - Chimeric Ab and humanized whole Abs had similar profiles with <1 - 13% antigenicity rates.
 - Some situations with long intervals between dosing (e.g., Remicade) may exacerbate serum sickness and reactions.
 - They saw more severe allergic reactions with longer intervals between dosing.
 - With Enbrel, they saw redness at injection site.

Ceredase: Excerpted from *Gaucher's News September 1993* interview with Dr. Richard Moscicki, Medical Director of Genzyme Biotherapeutics.

Biologics 2000 Conference: Immunogenicity stems from product purity (e.g., oxidized forms of IFN). Monoclonals are inherently antigenic; must be assessed for each Rx and cannot be compared across Rxs (e.g., HAMA, HACA, etc.). Some modified proteins (e.g., pegylated and fusion Rxs or Fc fragments) can be immunogenic. Clinical factors include: patient population, other illness, pre-existing Abs (e.g., rheumatoid arthritis (RA) abs), concomitant meds, dose and route of administration (e.g., SC is more immunogenic route than IV or IM injection). Immunogenicity measures are impacted by timing of assays too. Too early or too late and you won't see anything.

Types of antibodies to look for: binding Abs (IgM & IgG), neutralizing Abs (IgG) as seen in functional assays. For instance, with OKT3 (a transplant anti-rejection drug), 55-80% of patients had > 1000 Ab titer which interfered with efficacy.

Comparability studies for Factor IX (used in hemophilia Rx): assessment of plasma-derived Factor IX to the recombinant version (BeneFIX) used the hemophilia B dog model. Thrombogenicity is an issue stemming from impurities - the dog model gives them a standard tool for comparison. Fibrinopeptide A was measured after injection with Factor IX. Products were also compared in tolerated Hemophilia B dog model to assess tolerance to Factor IX. This was a useful tool to insert antigenically similar Rxs in the model and see what type of crossover reaction there was.



Immunogenicity (continued)

- **Interferon (IFN):**
 - Alpha IFNs: the IM, SC, and IV routes gave 0-25% neutralizing Abs;
 - Beta IFNs: SC gave 45% neutralizing Abs, but IM only 15%;
 - Gamma IFNs: SC had no neutralizing Abs activity.
- **Fibrinolytics:**
 - urokinase or tPA < 1% antigenicity
 - streptokinase varies with recent infection (1-4%).
- **EPO, G-CSF, or GM-CSF:**
 - Abs are rare
- **Interleukins:**
 - IL-2: binding Abs ranged 66-74% but neutralizing Abs were < 1%
 - IL-11: No neutralizing Abs seen

Fortunately, anaphylactic reactions to proteins are rare. Need to assess overall antigenicity - time to onset, duration, immunoglobulin profile and immunoglobulin shifts, binding vs. neutralizing, and potential impact on safety and efficacy.



Utility of Clinical Bridging Studies vs. Post-Marketing Surveillance Studies

- Fewer than 1% of all comparability studies require clinical studies
- Clinical bridging studies have limited utility in that they are too small to readily discern subtle changes and the impact on safety and efficacy. For instance, even a 0.3% adverse event rate would require a study of over 1,000 patients just to observe in 3 patients.
- FDA acknowledges this limitation and that is partly why they have such limited use; PK studies are more relevant when the measured blood level can be correlated to a therapeutic endpoint.
- More often, FDA requires specific post-marketing surveillance that may entail Ab assessments in all AE reports; long-term follow-up or other special assessments.
- Given this precedent, the multisource biotech firm may be in a better position to negotiate a small open-label confirmatory clinical trial for approval vs. a large multi-center study. Phase IV commitments would allow for assessment of other features FDA and the firm want.



Summary of Key Points

- Therapeutic equivalence criteria will probably be a mixture of innovator criteria + industry/ clinical experience with those classes of products. Your regulatory strategy will be best defined after an in-house comparison of analytical and bioassay data; what's next level of demonstrating therapeutic equivalence?
- Meet early with regulatory officials with data in hand; hypotheticals won't move project along in concrete manner. Have SAR data to support analytical comparability and links to bioassay, as well as links to safety and efficacy.
- Analytical equivalence must be established using the most current and holistic approaches. Data must be extensive to support bioassay variability, manufacturing flexibility, microheterogeneity, stability changes, etc.
- Manufacturing process controls must reflect product parameters well within 'edge of failure'. Manufacturing scale and process development changes must be supported by extensive comparability testing
- Stability data must be supported by extensive characterization testing to show subtle changes do not impact purity, potency, or safety. Stability testing should include innovator/ reference product as well as comparator lots



Summary of Key Points (continued)

- Product complexity will dictate the need for clinical trials; likely a certainty with any post-translational protein (MAbs, IFN, IL, etc.). Best to shoot for a small confirmatory, open-label study using surrogate endpoints - which may or may not be what the innovator used. Look at current clinical practice and FDA concurrence.
- Immunogenicity will likely be a component of almost all multisource biotech projects, but it is something to negotiate for post-marketing surveillance studies ... when you have SAR data and clinical history of product to show that Abs are not impacting safety and efficacy.
- Labelling that supports promotion and claims: Where substantive comparison data are part of the application, this should be allowed for safety and efficacy claims. However, FDA will not likely approve any claims of 'therapeutic equivalence' without direct clinical comparisons. For biologics, no AB rating, so substitution will be driven by cost.
- Comparative immunogenicity may be a touchy area in the labelling if there is no prior FDA and approval of those data.
- SAR databases can be used to expedite development via reduced analytical testing, preclinical testing, and clinical study design. There is ample precedent of innovator firms using this same feature to support initial approvals (e.g., Humulin, Avonex), as well as comparability protocols (e.g., t-PA, and vaccines).



List of References

- **FDA Guidelines, Guidances, & Points to Consider (PTC)**
 - CMC Information for a Therapeutic rDNA-derived Product or a Monoclonal Antibody Product for *in vivo* Use (August 1996)
 - PTC in the Characterization of Cell Lines Used to Produce Biologicals (1993)
 - PTC in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1994)
 - PTC for the Evaluation of Combination Vaccines: Production, Testing, and Clinical Study (1995)
 - PTC on Plasmid DNA Vaccines for Preventive Infectious Disease Indications (1996)
 - CMC and Establishment Description Information for a Vaccine or Related Product (1998)
 - CMC and Establishment Description Information for Human Blood and Blood Components Intended for Transfusion or for Further Manufacture (July 1998)
 - CMC and Establishment Description Information for Human Plasma-derived Biological Products or Animal Plasma-derived Products (Dec 1997)
 - Regulation of Placental/ Umbilical Cord Blood Stem Cell Products Intended for Transplantation or for Further Manufacture into Injectable Products (Dec 1995)
 - Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products
 - Stability Testing of Drug Substance and Drug Products (June 1998)
- **Code of Federal Regulations (CFR)**
 - 21 CFR 600 - 680
- **Federal Statutes**
 - Food and Drug Administration Modernization Act of 1997 (FDAMA)
 - Drug Price Competition and Patent Term Restoration Act of 1984 (Waxman-Hatch)
 - Food Drug & Cosmetic Act