



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



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



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)



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.



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.



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.



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



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.



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.



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.



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).



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.



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



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:**



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:** hypophysectomized 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



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.



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



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