

# Assessing Risk & Uncertainty in Biotech Development

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- Summary

The biggest risk companies often take is not planning for when the "risk" actually happens. That is, when speaking of risk vs. benefit ratios, it's important to anticipate worst-case scenarios. For instance, lots of contract manufacturing agreements don't elaborate on the details of who will pay for what when product is recalled by the FDA - even though it was initially manufactured correctly, passed all the release testing and is OK on stability. If FDA levies penalties against a BLA sponsor because their contract manufacturing firm wasn't operating under cGMPs, whose fault is that? The FDA will tell you it's the sponsor's fault. They are ultimately responsible for all cGMP aspects of production - regardless of third party procedures.

What happens when a process change is different and there is extensive comparability testing required? Will the contract manufacturer keep plugging away at a new process until the comparability is better ... or does the sponsor just have to eat the costs of making the 'apple' vs. 'orange' comparison work?

How does a firm handle an ethical crisis when the technology is remotely exploited for politically and religiously sensitive means? For instance, some firms use proprietary cloning technology that may be licensed to others for ethically sensitive areas. Does one try to control the technology or just articulate the limits of a firm's breadth to control it? How does one deal with the muddied waters of benefiting (financially) from some sales when they know the applications are politically contentious?

How does a firm protect itself from product liability suits ... even when the product is prescribed in the correct manner, no contentious off-label uses, and few serious, life-threatening adverse effects? What ethical standards are juries looking at in companies? What written policies are in effect ... and followed? How does a company deal with an errant salesperson or renegade marketing department?

# Purity

- Sensitivity of Methods
  - All applications must demonstrate strength, quality, purity, & potency; analytical methods must meet current standards of accuracy & reliability
  - Analytical method validation: accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, & robustness; follow the August 2000 guidance for details
  - Reference standards: characterization data for primary and working standards critical
  - Stability-indicating assay: required but sensitivity is negotiable depending on clinical phase
- Problem Areas
  - Out-of-specification (OOS) Data:
    - poor investigations,
    - acceptance of passing data when system suitability was NOT met (passing lots for market)
    - rejection of OOS data when system suitability WAS met (passing lots for market)
  - Impurities
    - New, unidentified impurities arising from finished product or stability samples with no investigation
    - Lack of mass balance
    - Lack of correlation of impurity levels with reduced potency
  - Shifts in Heterogeneity
    - Post-translational shifts in glycosylation patterns may occur spontaneously or in conjunction with minor manufacturing changes; critical to assess what potential impact on safety or efficacy
    - Are analytical methods sensitive enough to capture subtle changes that impact safety or efficacy?

The essence of this area is that companies will often do an adequate job of developing methods, but often don't do enough characterization testing over time to allow (1) adequate trending of enough production lots and (2) relationship of SAR to emerge. Some characterization studies may only entail a few lots post-change. Will cover handling OOS investigations in more detail in the analytical portion of the seminar.

## Potency: Bioassays

- **Potency requirement** for biologics means bioassay should be a quantitative measure of the biological activity - which can be linked to a functional aspect of efficacy or mechanism of action (e.g., binding + activation)
- **Establish sensitivity of bioassay to detect critical product parameters:** Can assay detect 10% aggregated, 5% oxidized, or 1% deamidated species? By using forced degradation stability, can do physico-chemical analysis and correlate to bioassay.
- Bioassays typically have **high RSD (or "noisy")** in terms of signal-to-noise ratio, **thus increased sampling** and testing to have confidence in absolute value. Also means more system suitability requirements needed.
- Some **compendial bioassays** (e.g., hypophsectomized rat femur growth for somatropin) **may be replaced by proprietary methods** - especially when compendial method is not specific or sensitive to detect subtle variations in product parameters
- **Gold standards:** need to use compendial-sourced reference standards for qualifying assay with your compound. May also use an innovator compound, but best to have a number of lots tested for process variability too.
- **Panels of tests:** sometimes, a single test is insufficient so you can combine several
- **Potency as a quantifiable unit:** need to express potency as a stated measure of activity/ml or gram. A relative measure to a reference standard is not likely to suffice.
- **Statistical Process Control (SPC):** sampling and continued analysis can help in long-term trending for manufacturing - which will help in comparability exercises

Bioassay controls: should have at least two controls - one below the expected concentration and one above the expected concentration, but both within range; exact concentrations are not necessary since the relative potency will be established in the assay. In choosing a potency assay, keep it as simple as possible: enzymatic vs. cell based vs. animal based. For animal assays, 5 animals per point OK for a "no" but 10 needed for a "yes"; expect annual fluctuations in animal responsiveness.

How well the bioassay correlates with in vivo activity is often a reflection of how well you know the mechanism of action. Better to have a robust potency assay that shows sensitivity than to have an imprecise assay that mimics the putative in vivo mechanisms.

Assay characterization will require: designating a standard, designating more than two controls, the sample and standard storage conditions, range, dilutional linearity, recovery, and precision.

NOTE: One of the most common failures of bioassays is the lack of dilutional linearity.

Precision: what are the results when at least three replicate assays are run with high, medium, and low potency material? As soon as assay development is done, need to start trending values for all assays run. **VIP to validate the assay before validating the process!** Practical considerations include: limiting dilutions to 1:100 or less; incubate the 96-well microtiter plates with more than 100  $\mu\text{L}$ /well; then run again with at least 150  $\mu\text{L}$ /well - to combat the misalignment of the plate reader and the "meniscus" artifact. Verify cell density is uniform in all wells ... before starting the experiment. Verify any positional effects early on (e.g., negative controls should be run in a variety of areas on the plate).

Also need to refer to latest USP chapter on bioassays.

## Potency: Glycobiology - Structural Diversity

- Structural diversity:
  - N-linked oligosaccharides: all contain a pentasaccharide known as "trimannosyl core" that may be further subdivided as:
    - Complex sugar type chains
    - High mannose sugar type chains
    - Hybrid type sugar chains
  - O-linked oligosaccharides
    - Fewer structural rules than N-linked sugar residues
  - Other glycoconjugates
    - Glycolipids: more than 200 glycosphingolipids known
    - Proteoglycans: glycosaminoglycans have much longer (100-200 residues) chains than O-linked or N-linked chains. High molecular weight structures with 6 major categories: hyaluronic acids, chondroitin 4-sulfates, chondroitin 6-sulfates, dermatan sulfates, heparan sulfates, and keratan sulfates. For example, heparin (discovered > 70 years ago) as an anti-coagulation material recently had a pentasaccharide structure identified that binds to anti-thrombin.
  - GPI (glycosylphosphatidylinositol) Membrane Anchors
    - Complex glycosphospholipids attached to a wide variety of cell membranes that appear to offer a stable association of protein with the membrane (see **Attachment 3**) for details.
- Structure-function Relationships
  - Examples of altered glycobiology and impact on SAR are detailed in **Attachment 3**.

See overview of glycobiology in **Attachment 3**.

## Potency: Glycobiology - Structure Function Relationships

- Activity may be:
  - **structural** (physical maintenance of tissue structure and integrity),
  - **protective** (no recognition by antibodies or proteases), or
  - **stabilizing** (involved in protein folding in the rough endoplasmic reticulum)
  - **organizational and barrier functions** (binding sites to fibronectin and collagen, which impacts phenotypic expression of cells)
  - **immune function** as binding sites for allergens, viruses, bacteria, parasites (e.g., influenza virus binding to sialic acid, cholera toxin to  $G_{M1}$  ganglioside, etc.). Indeed, binding to tissues is one of the leading factors contributing to pathogenicity of organisms.
  - Conversely, glycosylation can mask antigens or bind to available microbial sites as part of a **disposable "decoy."**
  - **"On-Off" switching** may occur with glycosylation, either via folding or antigen-antibody relationships, or the regulation of activity may be a "tuning" or modulation
  - Binding affinity of some glycosaminoglycans (e.g., heparin) can effectively **create a "depot"** by not allowing the bound proteins to diffuse into surrounding tissue, as well as protect the proteins from proteolysis - serving to create a depot of growth hormone in the extracellular matrix around growing cells that need it the most
  - **Intra-cellular trafficking** (e.g., mannose-6- $PO_4$  escorting new lysosomal enzymes to the lysosome)
  - **Inter-cellular trafficking** and "turnover" of proteins
  - **Hormonal actions** (e.g., immunosuppressive action of high-mannose chains)
  - **Cell-cell recognition**
- Given the diversity of biological functions, the only common feature among all is the mediation of 'specific recognition' events or modulation.

## Potency: Glycobiology - Points to Consider in SAR Interpretation

- Points to Consider in Interpretation of Structure-function Relationships
  - Genetic defects in tissue culture have limited consequences of the single cell and may need to be studied *in vivo* for true impact
  - Genetic defects (in glycosylation) are rare in intact organisms, but have highly variable consequences. This is most likely due to severe defects being lethal.
  - Clearance and biodistribution may be impacted by glycosylation patterns – as shown by duration of effect with pegylated interferon 2B $\alpha$  (e.g., PEG-Intron) or desialylated EPO or GM-CSF (granulocyte-macrophage stimulating factor). An inverse relationship exists for N-glycosylation site occupancy and biological activity; the glycoforms having the highest amount of glycosylation had the longest duration of biological effect (or slowest clearance).
  - Immunogenicity: since glycosylation can hide antigenic portions, antigenic epitopes may be hidden (or not) depending on the glycoform profile ... which is a key consideration of regulatory authorities right now. See detailed discussion in immunogenicity below, as well as in **Attachment 4**.

Glycoproteins from recombinant sources may have altered (or no) glycosylation, thus exposing novel antigenic sites. Masking of epitopes by oligosaccharides has also been demonstrated with influenza virus where an N-linked oligosaccharide prevented a MAb from binding to the H3 viral hemagglutinin protein chain. Absence of O-glycosylation on recombinant GM-CSF (from yeast) caused formation of serum antibodies directed against GM-CSF in 4 (out of 13) patients, but it wasn't clear if these antibodies were neutralizing. Inter-species variation in glycosylation patterns necessitates care in selection of cell type for expression of recombinant glycoproteins; humanized monoclonals are less immunogenic than classic murine hybridomas. While no clear evidence of clinical detriment from murine hybridomas, this could have larger impact for xenotransplantation.

## Potency: Glycobiology - Notable Activity in Other Areas

- Glycosylation activity also notable in:
  - Oncology:
    - markers on cancer cells, largely O-linked oligosaccharides can be used as targets for MAbs (e.g., Abs to sialosyl-Tn markers in colorectal cancer)
  - Inflammation:
    - selectins modulate adhesion of leukocytes to vascular endothelium, capillary morphogenesis, and lymphoid-endothelial cell interactions, particularly in sepsis;
    - Deficiency of terminal galactose on the Fc portion of the serum IgG antibody is associated with conditions like rheumatoid arthritis, Crohn's disease, tuberculosis, and ENL (erythema nodosum leprosum)
  - Infection:
    - Bacterial binding to epithelial cells mediated by carbohydrate-lectin interactions (e.g., *Helicobacter pylori* to surface of gastric epithelial mucosa; *Vibrio cholerae* toxin (B-subunit) binding to glycosphingolipid GM1(23), enabling A-subunit to enter cell and activate cAMP production)
    - Influenza virus binds to glycoconjugates on cell surfaces through HA (hemagglutinin) and a second surface enzyme - sialidase - allowing newly formed virions from infected cells.
    - HIV: glycoprotein (gp120) - which mediates binding to CD4 site - is heavily glycosylated with 24 relatively conserved N-linked units; gp160 also necessary for HIV function
  - Congenital disorders:
    - Dyserythropoietic anemia Type II (HEMPAS) caused by deficiency of Man $\alpha$ :N-acetylglucosaminyltransferase II enzyme
    - I-cell disease caused by a deficiency of enzyme phospho-N-acetylglucosaminyltransferase
    - Carbohydrate-deficient Glycoprotein Syndromes: a set of multi-systemic diseases with major nervous system involvement is linked to deficiency of asparagine-N-linked oligosaccharide

## Safety: Viral Inactivation/ Removal Validation

- Viral Inactivation/Removal Validation
  - Zero risk is a myth: Validation required since it's impossible to definitively show *absence* of virus particles (e.g., analytical method limitations, etc.).
  - Viral clearance: Validation based on multiple overlapping reduction/inactivation methods
  - Examples of product contamination
    - via incomplete inactivation such as vaccines (e.g., polio, rabies, and foot-and-mouth vaccine)
    - endogenous contamination with oncogenic viruses (e.g., SV40 virus in polio vaccine, avian leukosis virus [ALV] in yellow fever vaccine)
    - HBV contamination of serum used as stabilizer in yellow fever vaccine
    - Human Parvovirus B19 contamination of Factor VIII preparations stabilized with human albumin
    - Plasma derivatives as well as whole blood contaminated with HIV, hepatitis virus, parvovirus B19)
- Risk Assessment
  - How much is OK? Depends on product. Low levels in plasma products not allowed, but small residual levels ( $10^{-6}$  to  $10^{-9}$ ) in cell lines are deemed acceptable.
  - Goal is to evaluate each process step for known contaminants and estimate the robustness (of viral clearance) by characterizing its ability to clear model viruses.
- Risk Minimization
  - Three-fold: Screening starting materials, monitoring production, and evaluating production process
  - Generally, processes should be validated to remove 3-5 log orders of magnitude more virus than material estimated to be in starting material
  - Know the limits of model virus data: lipid enveloped viruses (e.g., HIV and hepatitis B) reduction does not ensure similar fate of non-enveloped viruses (e.g., hepatitis A and parvovirus B19)

Factors that impact viral method sensitivity include: sampling-associated logistical limits, masking of infectious virus by neutralizing antibodies in the plasma pool, extremely efficient amplification of viruses in cells substrates or product recipients, great diversity of viruses - hence necessity of doing specific assays for each virus, and unknown amphitropism of unknown viral variants. Thus, a negative PCR result is not conclusive proof of absence - since the sample size and assay sensitivity also weigh in. For example, a batch volume of 10L - using a 100 mL aliquot - with a viral particle load of  $10^6$ , you would get a negative PCR test (with a detection level of 100 viral particles/mL). See details in **Attachment 1**.

Continuous Cell Lines (CCL) are extensively characterized and while viral contamination will not be cytolytic, chronic or latent viruses may still be present. While endogenous retroviruses associated with CCLs are non-infectious they have oncogenic potential. Murine cell lines used in manufacturing monoclonal antibodies have potential rodent zoonotic agents, thus it's important to reduce the potential for latent virus particles or nucleic acid in the finished product. CHO cells can harbor contamination like Hantavirus. Humanized cell lines derived from B lymphocytes can harbor retroviruses, hepatitis, herpes virus, cytomegalovirus, and human papilloma virus - among others. Some cell line transformation is achieved using Epstein-Barr (infectious mononucleosis) virus or Sendai virus, which must be assessed. Since cell lines are often cultivated with bovine serum factors, those raw materials can be contaminated. Bovine viral diarrhea virus (BVDV) is the most common contaminant of bovine serum. Others include reovirus, infectious bovine rhinotracheitis virus (IBR), bovine leukemia virus, bovine parainfluenza 3 virus (PI-3), and bovine polyoma virus. Porcine parvovirus is a common contaminant of porcine trypsin used for cell culture preparation. Purification processes using monoclonal antibodies for purification (e.g., affinity chromatography) may also contribute viral contamination.

Removal can be via precipitation, chromatography (affinity, ion-exchange, gel-filtration), hydrophobic interaction (e.g., partition coefficients with solvent-detergents), or other methods using size, charge, density, binding affinities, etc. Inactivation can occur with pH effects, heat, radiation, sonication, or solvent-detergent (for lipid-enveloped viruses). Note that some inactivation methods can alter biological activity (and safety profile) of desired protein: changes in immunogenicity, production of neoantigens, or thrombogenicity may ensue, as well as loss of biological activity. Important to assess kinetics of viral inactivation since some heat-resistant or radiation resistant fractions may persist (e.g., cations have been shown to improve thermostability of viruses).

See **Attachment 1 - Viral Clearance Strategies for Biopharmaceutical Safety** - for more details of process development and assessment. However, see CD ROM - The Library/Viral Validation for more details and copies of guidances.

## Safety: BSE/TSE - Background

- TSE/BSE (Transmissible Spongiform Encephalitis/Bovine Spongiform Encephalitis)
  - Prions, which are putative agents of fatal neurodegenerative conditions such as Creutzfeldt-Jakob Disease (CJD), variant CJD (vCJD), Gerstmann-Strausler-Scheinker (GSS) disease, kuru, and fatal familial insomnia (FFI); clinical latency for genetic CJD may be up to 30 years.
  - Potential for transmission via blood, transplanted tissue, human cells or other cell-based products (HCT/Ps)
  - Common feature of all TSE is the conversion of a host-encoded sialoglycoprotein to a protease-resistant isoform as a consequence of infection
  - Only pathognomonic marker is abnormal isoform Pr<sup>Pres</sup> - a host-encoded, protease sensitive glycosylphosphatidylinositol-anchored cell surface protein - noted as Pr<sup>Pc</sup>
  - Variant CJD (vCJD) differs from familial CJD in that:
    - vCJD patients are younger (median age 28 years),
    - have psychiatric and sensory symptoms early in the course of the disease,
    - do not have EEG abnormalities,
    - have a longer duration of illness (median survival is 13 months compared to 4 months),
    - have neuropathologic features with florid prion plaques surrounded by spongiform changes, and
    - immunohistochemistry shows abnormal prion protein detected in lymphoid tissues.

See (1) a 4-part article series, (2) FDA June 2002 guidance on preventative measures, and CPMP May 2001 guidance (**Attachment 2**); additional information also on CD ROM - The Library/BSE/TSE folder. Contamination became widespread through the use of medicated animal feeds that were comprised of bone and other tissue from infected animals. Although the practice is no longer practiced in US/Canada, UK, Europe, and Australia ... it may still be done in other nations. Importation of beef from suspect regions is based on (1) history of infection and (2) monitoring abilities.

## Safety: BSE/TSE - Donor Deferral Guidance

- Donor Deferral Criteria for **CJD**:
  - Known diagnosis of CJD or family history
  - Patients who received injections of human pituitary-derived growth hormone and
  - Patients who had dura mater transplants and/or cornea.
  - lived in the UK/Europe for extended periods during the 1980s and 1990s
- Donor Deferral Criteria for **vCJD**:
  - Exposure to BSE agent from British beef in the UK
  - Exposure to BSE agent from British beef products distributed outside the UK during the BSE epidemic (prior to implementation of food control measures in 1996)
  - Exposure to BSE agent from infected cows in the country of residence ("indigenous BSE") outside of the UK
  - Theoretical exposure to vCJD agent by transfusion of blood or blood products from UK donors
  - Theoretical exposure to BSE agent through bovine insulin produced from UK cattle
- **CPMP Guidance (May 2001)** notes four categories of risk associated with tissues:
  - Category I: brain, spinal cord, eye
  - Category II: ileum, lymph nodes, proximal colon, spleen, tonsils, dura mater, pineal gland, placenta, CSF, pituitary, adrenal
  - Category III: distal colon, nasal mucosa, peripheral nerves, bone marrow, liver, lung, pancreas, thymus
  - Category IV: blood clot, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicles, serum, skeletal muscle, testis, thyroid, uterus, fetal tissue, bile, bone, cartilage, connective tissue, hair, skin, urine.

See FDA guidance in **Attachment 2** for details regarding each risk category and background information.

## Safety: BSE/TSE - Chemical Resistance

- Resistance:
  - Formalin (0.5%) up to 4 months
  - Alcohols
  - Alkylating agents:  $\beta$ -propiolactone, ethylene oxide, glutaldehyde, formaldehyde)
  - Phenolic disinfectants
  - Potassium permanganate (0.2% up to 16 hours)
  - pH 2.0 - 10.0
  - Dry Heat:
    - 160°C for 24 hours (no effect)
    - 360° C for 1 hour - infectivity still remains
  - Autoclaving:
    - 126° C for 1 hour at 15 psi - infectivity remains
- Susceptibility
  - Autoclaving:
    - 134°-138° C for 18 minutes hour at 30 psi
    - 132° C for 1 hour at 30 psi
  - 2% Sodium Hypochlorite (bleach) for 1 hour
  - Sodium hydroxide:
    - 0.1 N NaOH (pH 13.0 - a  $10^6$  log reduction)
    - 1 M NaOH (pH 14.0) >  $10^6$  log reduction

## Efficacy: Correlation to SAR (Structure-Activity Relationship)

- SAR is linked early on to a process scale-specific material that is used for preclinical, stability, and early clinical studies. As process development evolves, the comparability exercise widens and the correlation - or lack of it - is defined.
  - For example, a synthetic peptide is made at one site but then moved to another site for cGMP production. Although they are virtually identical in physico-chemical ways, the cGMP site material is 10X-less potent in the bioassay. What's different; what's missing in this assessment?
  - For one, are the observed bioassay differences a reflection of (1) altered receptor binding affinity or (2) same affinity but altered receptor/bioassay activation? To determine the difference, one should look at receptor kinetics (e.g., Schild plot, competitive binding assays).
  - You can also assess the sensitivity of the bioassay to impurities by doing forced degradation of API and drug product and testing that. Using a single vial, dilute into several stock solutions that can be subjected to heat, cold, freezing, pH (acid and base), light, photolysis, and oxidation via a 3% hydrogen peroxide solution. Using these varied conditions over a range of hours to weeks, then subject them to intensive characterization testing and correlate the physico-chemical profile with the sensitivity of the bioassay.
- Bioassay activity and physico-chemical profile should be correlated with purported mechanisms of action for *in vivo* efficacy. For instance, (preclinical) vaccine antibody profiles should not change spuriously. If they do, what does this suggest about shifts in the antigen or antigen presentation?

There are countless examples of SAR links to efficacy - so much so that this became the underlying premise for comparability protocols in the early 1990s. Although a biotech product reflects the process, it's possible that some well-characterized or specified biotech products could be analyzed and correlated much the way drugs were - thus affording greater latitude in manufacturing changes.

Surrogate endpoints derived from primary efficacy endpoints of morbidity or mortality that took too long to assess in chronic disorders or became unethical delays in life-threatening conditions. The development of primary efficacy endpoints also allowed surrogate endpoint validation of other parameters. Some examples include:

- Geometric Mean Titers (GMT) and seroconversion rates in vaccine development (vs. confirmation of prophylaxis rates)
- Patency, cardiac output, diagnostic imaging, and quality of life in fibrinolytic use for thromboembolic disorders [cardiac infarction/ pulmonary emboli] (vs morbidity rates)
- Pulmonary function testing in cystic fibrosis, reduced IV antibiotic use, reduced ICU (vs. morbidity)

## Immunogenicity: Potential Risks

- Immunogenicity Reactions (per Gel-Coombs Classification):
  - **Type I:** immediate hypersensitivity (e.g., penicillin allergy)
  - **Type II:** cytotoxic (e.g., acquired hemolytic anemia by methyldopa or penicillin; thrombocytopenia induced by quinidine)
  - **Type III:** immune complex (e.g., penicillin, sulfonamides, thiouracil, and phenytoin)
  - **Type IV:** cell mediated (e.g., contact dermatitis, delayed hypersensitivity reactions)
  - **Miscellaneous Syndromes:** hypersensitivity vasculitis, anti-convulsant hypersensitivity, pulmonary drug hypersensitivity, immunologic hepatitis, blistering disorders, pseudo-allergic reactions, non-immune drug-related reactions
- Potential risk factors include:
  - Structural nature of compound
  - Genetic predisposition to hypersensitivity for products of that class
  - Drug product formulation
  - Route of administration
  - Frequency of administration
  - Historical safety record of other compounds in that product class
  - Lack of standard assays make comparisons of immunogenicity profiles within a product class difficult
- Consequences include inhibition of product function, altered PK, auto-immune syndromes, circulating immune-complex (CIC) formation/deposition, allergic reactions

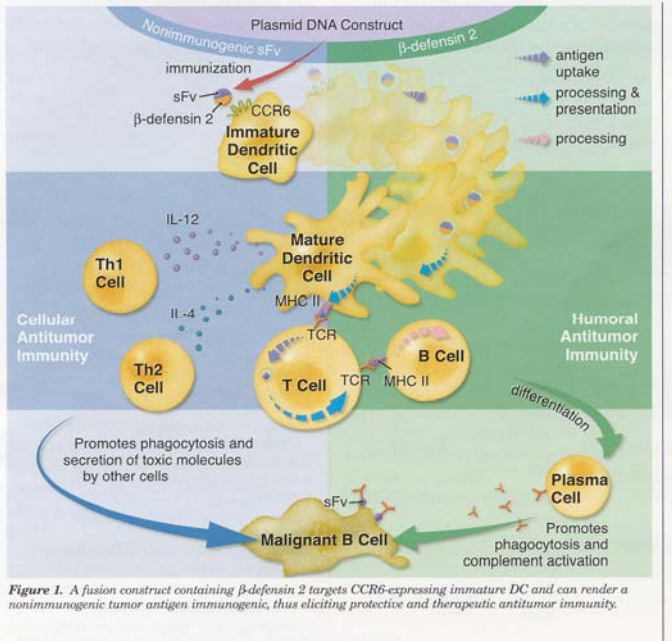
**Type I reactions** mediated by IgE with symptoms ranging from anaphylaxis to rash, swollen throat with difficult swallowing and breathing, cardiopulmonary complications. Most common cause are antibiotics, but others include insulin, enzymes (streptokinase), equine antisera, murine monoclonal antibodies, protamine, and heparin. Type I reactions also noted after exposure to excipients like eugenol, carmine, vegetable gums, paraben, thiomersal, sodium metabisulfite, formaldehyde, and sulfonechloramide.

**Type II reactions** are complement-mediated via IgM or IgG antibodies formed in response to altered cell surface. Thrombocytopenia from drug-induced immune reactions is well documented, such as seen with quinine, quinidine, acetaminophen, propylthiouracil, gold salts, and sulfonamides. Platelet damage is mediated primarily by drug-immune complexes absorbed onto platelet membranes. Granulocytopenia may also develop in reaction to pyrazolone derivatives, phenothiazines, thiouracils, sulfonamides, and anti-convulsants. Although rare, some responses have long-lasting consequences; for instance, antibodies to recombinant TPO [thrombopoietin] cross-reacted with endogenous TPO, which led to autoimmune dysfunction and irreversible thrombocytopenia.

**Type III** mediated by immune complexes formed by slight antigen excess; symptoms include fever, rash, swollen lymph nodes, and stiffness - which appear 1-3 weeks after exposure and subside when drug is completely eliminated. Serum sickness was originally noted with use of equine antitoxins/antivenins for passive immunization. Drugs with Type III reactions include penicillin, sulfonamides, thiouracils, and phenytoin. Most of the immune reaction is mediated by IgG and IgM, but IgE mediates the urticarial lesions seen early in the disease state.

**Type IV reactions** are mediated by CD4+ cells and/or CD8+ cells; symptoms include contact dermatitis and delayed hypersensitivity reactions evidenced by elevated cytokine levels, fibrosis, lymphocytic infiltrates, disseminated granulomata - akin to graft vs. host reactions maintained by T cells. Drugs involved include topical formulations of penicillin, local anesthetics, and antihistamines.

# Immunogenicity: Mechanisms of Action



**Figure 1.** A fusion construct containing  $\beta$ -defensin 2 targets CCR6-expressing immature DC and can render a nonimmunogenic tumor antigen immunogenic, thus eliciting protective and therapeutic antitumor immunity.

## Immunogenicity: Predicting Activity & Development Strategies

- Predicting immunogenicity is difficult
  - Animal models have limited utility since all human proteins will be antigenic
  - Transgenic animals offer potential with "humanized" immune systems
    - Animals raised with transgenic human proteins recognize the native human peptide as normal. Changes to the protein structure that alter immune profiles in these animals may well be predictive of human reactivity.
    - Transgenic mice can express human immunoglobulins and human MHC (major histocompatibility) Class II genes
  - Assessing the immune response in a transgenic model allows profiling of antibody response
    - What titers of binding antibody present?
    - What's the immunoglobulin profile (e.g., IgG, IgE, etc.) of the response?
    - Are they neutralizing antibodies?
    - How does the immune response change over time with single dosing? Repeated dosing? Low doses? High doses?
    - Is tolerance observed?
    - Is the method sensitive enough to allow epitope mapping and T-cell epitope removal?
- Development Strategies:
  - What is degree of sequence homology to native protein?
  - Do assays detect aggregates with sufficient sensitivity?
  - Assess process and controls to assure consistency and reproducibility in glycosylation.
  - Use bioinformatics and transgenic animal models to identify antigenic epitopes.
  - What is immune response profile and how rate-limiting to therapy is it?
  - Do antibodies impact PK or correlate with adverse events?
  - Consider monitoring immunogenicity profiles in at least 200 patients for > 6 months.

See detailed discussion in **Attachment 4**. With greater discussion of multisource biotech, the topic of immunogenicity has been raised numerous times as a call to full clinical program; that no biotech product is so well characterized it can be marketed without a full study of these phenomena. However, the relevance of safety in immunogenicity is still being debated. Some products (e.g., recombinant plasma derivatives, monoclonal antibodies, etc.) that are highly immunogenic - to the point of having neutralizing antibodies negate or diminish the clinical effect - are on the market and the innovator does not do extended immunological comparisons prior to marketing these updated versions. Other products (e.g., IGIV, growth hormone, insulin, etc.) do not have a marketing history (or clinical use history) of immunogenicity, so the value of assessing these aspects becomes academic. In order to derive a more balanced approach, it's necessary to evaluate the complete history of reported drug-related allergies and other immunologically related phenomena to better assess the true picture.

For additional information, also see **Assessment of Immunotoxic Potential of Human Pharmaceuticals: A Workshop Report** in *DIA*, Vol. 36: 417-427, 2002 - which includes references to the **CPMP July 200 Note for Guidance on Repeated Dose Toxicity Testing** (which included immunotoxicity assessments). For instance, one recommendation of the workshop was that this type of testing could be performed in Phase II in parallel with clinical trials (as opposed to a prerequisite or parallel cohort during Phase I studies). Key workshop conclusions also noted functional testing is a requisite with a determination on response with T-cell dependent profiles, which should be included in routine screening.

## Production-related Areas: Process Validation & Comparability

- Process Validation
  - Based on process development data; shows process is consistent, reproducible and that resulting product meets the finished product & stability criteria; comparability assessment needed for significant changes and/or scale-up
  - Process validation for biologics must be complete at proposed commercial scale and summarized in the BLA - unlike NDAs, where commercial scale validation can wait after NDA filing but before market launch. Timing to start validation is critical.
  - Aseptic process validation problems: (1) poor investigations of exceeded limits and thresholds, (2) positive results in sterility testing, (3) environmental monitoring (EM) program is inadequate to confirm control, (4) HVAC and Water for Injection (WFI) systems not validated.
  - Companies will release product made outside validation parameters based on limited finished product testing.
- Comparability
  - Concept applied to post-approval changes, but once you have started pivotal efficacy (e.g., Phase III) trials - you need it to bridge clinical material to proposed marketed material (if different scale, process parameters, raw materials, etc.).
  - Also need it throughout development to show preclinical testing (e.g., impurities, SAR, etc.) is still supportive of proposed commercial scale material. Otherwise, may need to repeat some early studies.
  - See CD ROM: The Library/CBER/Comparability of Biotech Products and Drugs for copy of comparability guidances and related documents

Scale of Manufacturing: Pilot vs. Commercial

Process Development: Neural Nets

Process Development: Edge of Failure

Process Validation

Statistical Process Control (SPC): Sampling

Links: Impact of Manufacturing Controls to -

Analysis of Product Characteristics: Comparability testing

Surrogate Equivalence: Impact on activity

Stability: Emergence of undetected impurities

Analytical Methods: Consistency of methodology/ data

Setting Rational Specifications

## Production-related Areas: QSIT

- QSIT (Quality Systems Inspection Technique)
  - FDA's latest approach has been to examine quality systems (see diagram on following page) that reviews relationships of systems (e.g., records and documents, production, facility, corrective action, etc.)
  - FDA issued QSIT guidance in August 1999 (see CD ROM: The Library/Auditing & Quality Assurance Programs/Guide to Inspections of Quality Systems for copy of guidance).
  - Key compliance problem areas include:
    - (1) change control,
    - (2) lots released for market by unapproved changes,
    - (3) no lot accountability for which lots were manufactured by which SOPs or process criteria, etc.,
    - (4) failure to review and approve procedures,
    - (5) failure to qualify computers,
    - (6) poor state of control for packaging and labelling operations

## Production-related Areas: Change Control

- Team Biologics is a system-wide evaluation of procedures and documentation and how changes are documented and implemented.
- Using an electronic tracking system allows easier document management of specifications and manufacturing change controls over time (see production flowchart map).
  - Ease of tracking helps identify problem areas (e.g., gap analysis) and avoid AIP (Application Integrity Policy) issues.
  - Ultimate product is a transparent document management system - on a company intranet - that allows rapid, real-time assessment of specification and manufacturing change controls, regulatory filing status, and implementation.
  - Software + personnel (2-3 full-time) = approx. \$100 K/year versus cost of a small product recall = approx. \$1 - 5 million. Cost difference ranges from 10 to 50-fold more.
- Examples of system failures include:
  - Manufacturing biobatch lots or NDA/ BLA stability lots before analytical method validation is complete.
  - Manufacturing ANDA demonstration lots and testing for release prior to completion of technology transfer for analytical methods.
  - Performing OOS investigations on IND lots because the investigational product was mistakenly tested per the specifications for marketed product, not the wider IND product specifications.
  - Release and sale of marketed product made by unapproved changes.
  - Implementation of new procedures without adequate training in place.
  - Release and sale of marketed product with incorrect labelling.
  - Scale of manufacture changes without appropriate process validation (e.g., expanded powder filling operations).

### EXAMPLES OF CHANGES CLASSIFIED UNDER 601.12

- Change from manual monitoring to Critical Utilities Network (CUN) - Computer Control and monitoring of HVAC supply (Environmental Monitoring and Pressure Sensors). CBE-30.
- Replacement of a lyophilizer control system (same operating principles). AR.
- Modifications to an existing WFI system - removal of inactive drops and corresponding piping and addition of additional piping at the same time; more piping is removed than added. CBE-30.
- Installation of a new pasteurizer in a previously non-licensed area. Pasteurizer is of similar design to existing model. CBE-30.
- Addition of a new WFI sub-distribution system. CBE-30.
- Change in Sanitization practices including change in sanitization solutions. AR
- Eliminate the Post wash of an Ultrafilter and addition of the post wash to product when the purpose of the Post wash was a yield recovery step which was determined to be minimal and did not warrant the additional processing time. AR
- Change in the cleaning agent used for CIP of equipment. CBE-30
- Change of a Cassette UF Process that was validated and licensed for the diafiltration to include 6 volumes of high salt buffer and 3 volumes of low salt buffer to 3 volumes high salt and 3 volumes low salt. The purpose of the diafiltration is to reduce the alcohol level to below specification. The validation data demonstrates satisfactory removal of alcohol after 6 volume exchanges. CBE-30
- Change the Post wash step at the end of final concentration from [600 Kg of WFI recirculated through the cassette UF and transferred for further concentration to a 700 sq. ft. UF.] to [50 Kg WFI transferred once through the cassette UF to the product (eliminate use of the 700 sq. ft unit)]. CBE-30
- Extension an incubation time of the product with sodium thiocyanate. (A viral reduction step validated and licensed at X hours.) Extension of the hold time will not impact the viral reduction capabilities. Quality of product is not impacted by extending the hold time. PAS?

## Ethical Considerations

- Use of fetal embryonic tissue versus established cell lines or adult stem cell lines
- Use of animal populations for either transgenics or xenotransplantation
- Use of animal colonies for particular hybridoma or ascites production
- Applications of fertility technology and potential overlap of human cloning
- Safety considerations for novel technology
- Plant transgenics: food production (e.g., Frankenfoods)
  - StarLink's contamination of 430 million bushels of corn became a cautionary tale of the controls being inadequate
  - Monsanto's development of pesticide resistant strains wreaked havoc on endogenous Monarch butterfly populations
  - Political activists lump the IMF (International Monetary Fund) and WTO (World Trade Organization) as the "Death Stars" behind manipulation of developing nations and as 'pushers' of the intellectual property behind bio-engineered foods.
- Plant transgenics: medicine production & potential cross-contamination
- Pharmacogenomic data used for medical screening and bias: personal privacy issues and insurance bias

## Summary

Late on the third day, at the very moment when, at sunset, we were making our way through a herd of hippopotamuses, there flashed upon my mind, unforeseen and unsought, the phrase, "Reverence for Life."

*Out of My Life and Thought* (1949)  
Albert Schweitzer