

# Critical Quality Attributes Pertaining to Immunogenicity: A Preponderance of Evidence Approach to Control Strategies

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# Quality Target Product Profile (QTPP): A *Sine Qua Non* for Clinical Performance



**ICH Q8(R2) QTPP Definition:** A **prospective** summary of the quality characteristics of a drug product that **ideally** will be achieved to ensure the desired quality, taking into account safety and efficacy.

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graph TD; A[ICH Q8(R2) QTPP Definition: A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy.] --> B[QTPP: quality characteristics to ensure safety and efficacy as promised in the label];
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**QTPP:** quality characteristics to ensure safety and efficacy *as promised in the label*

# Elucidation of Quality Target Product Profile Requires Determination of Critical Quality Attributes

## Critical Quality Attribute

Definition: A physical, chemical, biological, or microbiological property *or characteristic* that should be within an appropriate limit, range, or distribution to ensure the desired product quality (ICH Q8)

Designation: by definitive data from clinical sources or a preponderance of evidence from several sources including clinical, in vitro, and animal data, and prior knowledge including published information of impact on PK/PD, potency, immunogenicity, and safety

# Considerations for Establishing CQAs in Quality Risk Management Exercise: Guidance for Industry

## Q8, Q9, & Q10- Q&A



- Focus is on patient protection:
  - “the protection of the patient by managing the risk to quality should be considered of prime importance”
  - scientific rationale and quality risk management processes are used to reach a conclusion on what are critical quality attributes and critical process parameters for a given product and process
  - quality attribute criticality is based primarily on *severity of harm* and does not change as a result of risk management
  - the level of effort, formality, and documentation of the quality risk management process should be commensurate with the level of risk.
- Process parameter criticality
  - Linked to parameter’s effect on any critical quality attribute: can change as a result of risk management

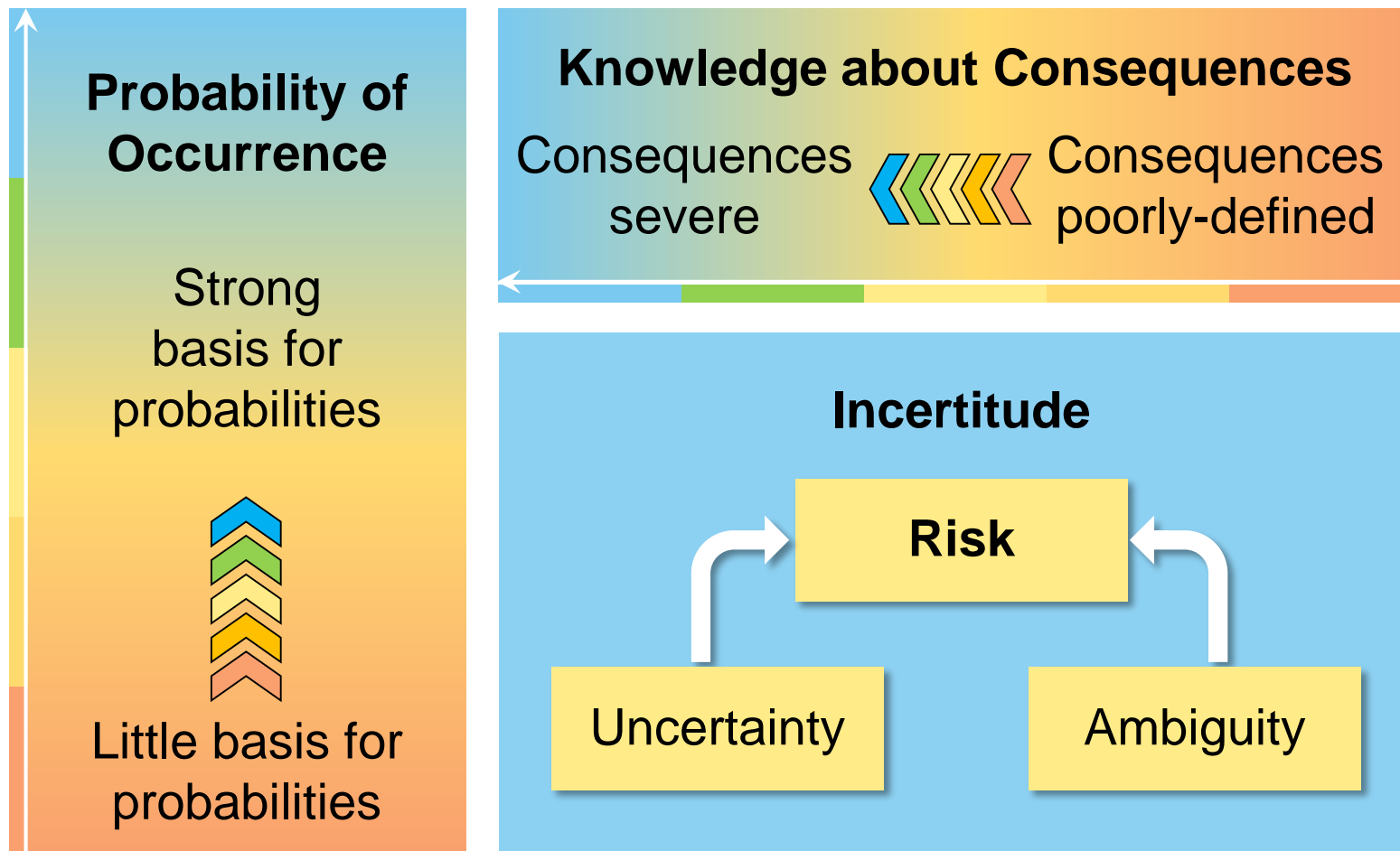
# Product Understanding: Determining CQAs



- Attributes are assessed for clinical impact as the severity of harm to safety and efficacy: the range of a candidate CQA is evaluated in different batches and scored for impact on activity (desired effect), PK/PD, safety, and immunogenicity
- Attributes are ranked based on *strength of data* used to assess impact and *extent of residual uncertainty* either in consequences or likelihood
- ***Impact and residual uncertainty*** determine where an attribute is on the criticality continuum
- Process capability and detectability should not be considered as primary drivers in the attribute risk assessment, but should be considered in the final control strategy

# Assessment of Risk Tempered by Uncertainty

(modified from Stirling and Gee 2002)



# Managing Uncertainty: A Perspective on Risk Pertaining to Product Quality Attributes as They Bear on Immunogenicity of Therapeutic Proteins



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- When there is a strong scientific basis for a PQA to be a CQA with potential to affect safety and efficacy, but insufficient basis for assigning probability of occurrence, the attribute should be assumed critical to the observed S&E profile
  - Criticality and need for control strengthened by a preponderance of evidence supporting potential impact on clinical performance
  - Control strategy, including attribute acceptance criteria, needs to include greater consideration for manufacturing process capability and analytical capability: acceptance criteria should be as tight as capability practically allows
- Consistent with the “precautionary principle”: rather than presume that specific substances are safe until proven dangerous, the precautionary principle establishes a presumption in favor of protecting the public health in the face of uncertainty.
- Accumulate sufficient data (clinical, nonclinical) to demonstrate or disprove criticality to S&E



# Assurance of Desired Quality Target Product Profile Requires a Robust Control Strategy

- A control strategy is a planned set of controls, derived from current product and process understanding, that assures process performance and product quality
  - To deliver consistent product quality and minimize risk to patient safety and product efficacy.
  - To generate process and product understanding and identify sources of variability.
  - To provide an opportunity to shift controls upstream and minimize the need for end product testing.
  - To support the control of the process such that the variability (e.g., of raw materials) can be compensated for in an adaptable manner
  - Consideration should be given to improving the control strategy over the lifecycle: in response to assessment of data trends over time and other knowledge gained (Guidance Q8-10 Q&A). Product approval based on limited numbers of product lots and usually small numbers of patients.

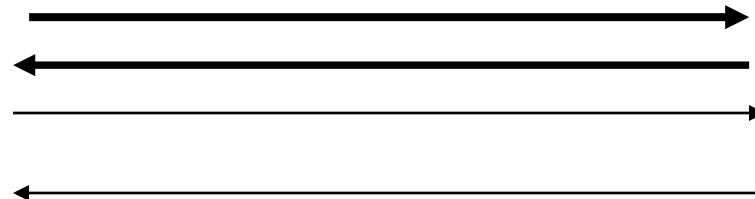
# Control Strategy Development: Understanding CQAs and Impact of Process on CQA Control



Enhanced approaches  
Increased product and process understanding

## CQAs

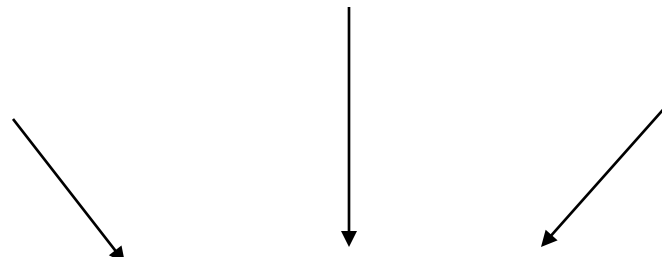
A preponderance of evidence from several sources or definitive data from clinical sources: in vitro animal data, clinical data, and prior knowledge including published information of impact on PK/PD, potency, immunogenicity, and safety



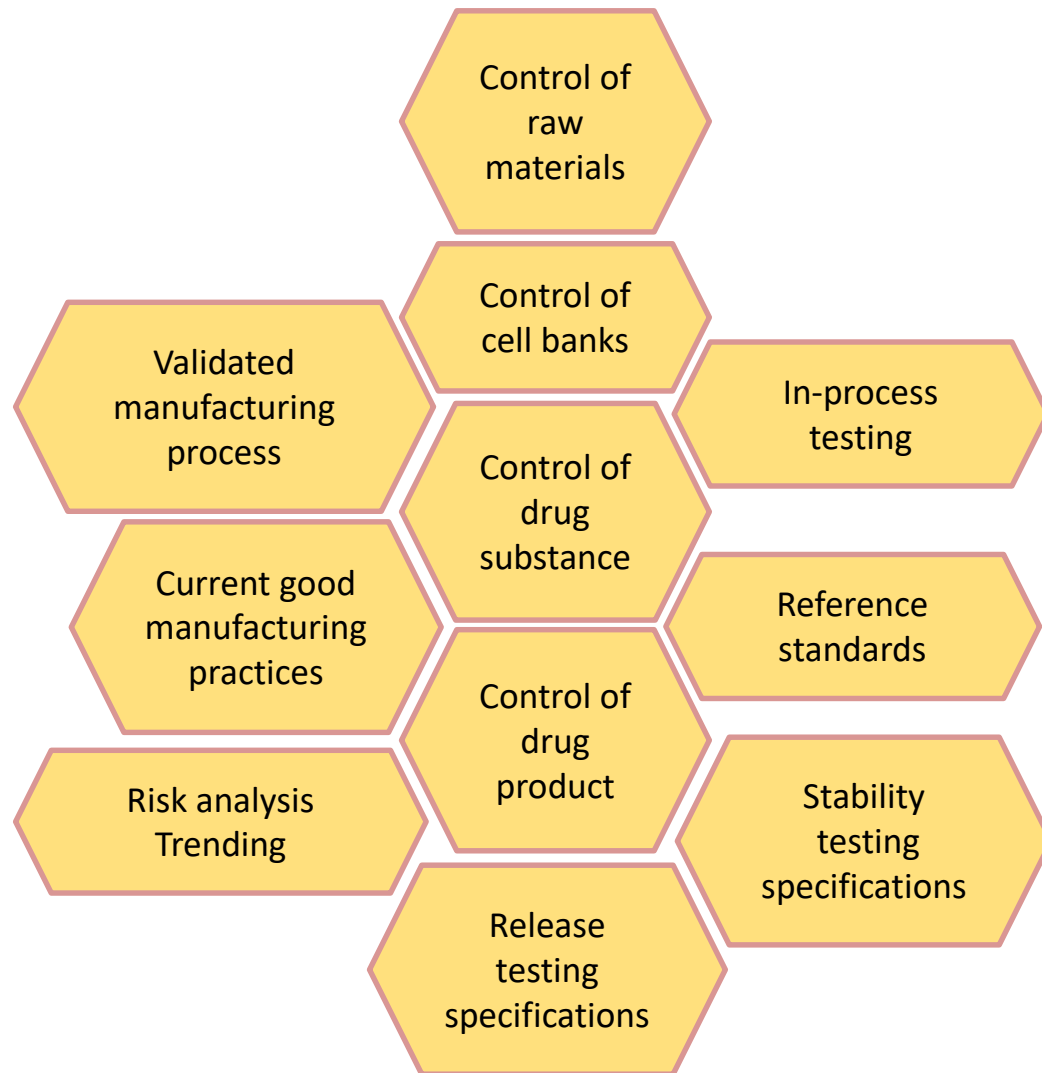
## Process Development and Characterization

Process characterization studies to determine how CQAs are influenced by the manufacturing process and material attributes

Control Strategy



# Control Strategies for Biotech Products



# Levels of Control: Overarching Considerations for Control of CQAs

- Determine how well controlled a CQA is by the manufacturing process.
- Validation of removal of CQAs with potential negative impact on product performance (eg DNA and HCP) may eliminate need for specific attribute testing
- Testing and elucidation of specifications should be done for CQAs that may change over time or, minimally, after the last manufacturing step in which they could be impacted
- Elucidate the effects of process parameters and material attributes on CQAs. These studies also define ranges for process parameters

# CQAs that Bear on Immunogenicity

- CQAs that are “Characteristics”
  - Protein origin: foreign vs self (with qualifications)
  - Abundance of endogenous protein counterpart of therapeutic
- CQAs that are product attributes assessed in therapeutic protein products
  - Protein structure:
    - primary structure including sequence divergence or polymorphisms
    - higher order structure-aggregates
  - post-translational modifications/chemical degradation
- Impurities: process and product related
- Immunomodulatory properties of the protein therapeutic: immunostimulatory vs immune suppressive

# Immunogenicity Risk Assessment: Consequences for Safety

- **Fatality/Severe Morbidity**
  - Anaphylaxis: clinical definition, does not imply mechanism
    - Proteins of non-human origin, eg, aprotinin, asparaginase
    - Replacement human proteins in knockout phenotype: eg, Factor IX in hemophilia B
  - Therapeutic Counterparts of Endogenous Proteins
    - Cross reactive neutralization of endogenous factor with non-redundant function resulting in deficiency syndrome
    - Cross reactive binding and activation of endogenous receptor with cytokine release syndrome by antibodies to homologous therapeutic receptor product
  - Immune Complex Mediated Disease: delayed hypersensitivity
    - Serum sickness; nephropathy
    - Most often seen when high doses of therapeutic proteins are administered in setting of a sustained high titered antibody response

# Immunogenicity Risk Assessment

## Consequences for Efficacy



- **Fatality/Severe Morbidity**
  - Neutralizing antibodies to life saving therapeutics :
    - Enzyme and Coagulation Factor Replacement Therapies
  - Diminished efficacy of highly effective therapeutics
    - mAbs: eg TNF blockers
- **Alterations in PK**
  - Antibodies to protein therapeutics may diminish or enhance PK
  - Sustained or increased anti-drug antibody in the face of continued or escalated treatment dosage/frequency of product may lead to
    - epitope spread and generation of neutralizing antibodies
    - immune complex disease
- **No apparent effect**
  - But sustained response may lead to epitope spread and generation of neutralizing responses
    - IL-2
    - IFN- $\gamma$

# CQAs and Impact on Safety and Efficacy: Examples



# Aggregates: Pure Red Cell Aplasia in Response to rhuEPOs

- Endogenous Erythropoietin:
  - sole factor mediating red blood cell production
  - low abundance protein; levels in nanomolar range (sea level); spikes in levels due to changes in oxygen levels: altitude changes, anemia.
- Tolerance relatively robust: few cases of PRCA related to Epo usage prior to 1998: associated with autoimmune disease
- Increased incidence in PRCA following changes in formulation, container closure and administration route in 1998

# PRCA in Development of Biosimilar Epo: Suspect Lineup in the Search for the Smoking Gun

- Three cases of NABs and one case of PRCA in development of a biosimilar Epo: *two implicated batches* thoroughly analyzed for suspect PQAs
  - aggregates
    - Micelles of erythropoietin: polysorbate generated micelles
    - *Tungsten leachates* from tungsten used in needle hub formation: protein aggregation from tungsten oxides; shown previously to aggregate other therapeutic proteins
  - adjuvant material leaching from rubber stopper:
    - *vultac* responsible for cross linking of rubber protein; can cross link other proteins eg epo? no
    - vultac has adjuvant properties (Sharma et al 2004)

# Tungsten Mediated Aggregates Likely Root Cause of Induction of Epo Antibodies in Biosimilar Epo

(Seidl A et al 2012)

Potential risk factor	Suspect batches had higher levels than			Literature available to suggest a role in immunogenicity	Involvement in current root-cause
	Other batches of study drug	Other batches not used as study drug	Reference product (Eprex®)		
<b>Drug product</b>					
<b>Aggregation</b>	<b>Yes</b>	<b>Yes</b>	<b>No</b>	Yes	<b>Most likely</b>
<b>Unfolded variants/ irreversible Dimers</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	Yes	<b>Most likely</b>
Subvisible particles	No	No	No	Yes	Unlikely
<b>Inorganic leachates</b>	<b>Yes (tungsten)</b>	<b>Yes (tungsten)</b>	<b>Yes (tungsten)</b>	Yes*	<b>Most likely</b>
<b>Organic leachates</b>	<b>No</b>	<b>No</b>	<b>No</b>	Yes	<b>Highly unlikely</b>
Oxidation Met-54	No	No	No	No	Highly unlikely
Oxidation Trp-64	No	No	No	No	Highly unlikely
Deamidation/aspartate	No	No	No	Yes	Highly unlikely
Isomerization					
Degradation	No	No	No	Yes	Highly unlikely
Silicone oil	No	No	No	Unclear	Highly unlikely
<b>Polysorbate 80 micelles</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>Highly unlikely</b>

\* Role in protein aggregation

# Increased Tungsten ( $\Omega$ ) Content Unique to Implicated Product Batches

Batch	770307	780307	260108	270108	400208	770508	770508	140808	700106	8ESTE00	770508	750906	380409	060609
Sample type	RSM	RSM	CSS	CSS	CSC	RSM	CC6	Ref. sample	Placebo	Erypo® Ref.	RSM	Ref. sample	Ref. sample	Ref. sample
$\Omega$	<LOD	<LOD	0.99	0.97	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.52
Al	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Si	2.32	1.57	6.42	3.20	3.70	1.86	3.26	3.17	17.20	29.50	1.86	2.14	2.03	1.55
Ca	<LOD	0.52	0.78	<LOD	1.31	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Se	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.23	<LOD	<LOD	<LOD	<LOD	<LOD

# Criticality of Traceability

(EMA Guideline on Immunogenicity Assessment of Biotechnology Derived Therapeutic Proteins 2015)

- “Identification of the product responsible for an adverse event, traceability, is important for biopharmaceuticals. This is especially important for adverse events related to immunogenicity. Traceability is important for both routine pharmacovigilance (collection of spontaneously reported adverse events) and additional pharmacovigilance activities. Appropriate measures to improve traceability, collection of brand name and batch number, should be taken.”

# Tungsten Oxides, Aggregates and SVPs: Control Strategy

- Manufacturing process removal evaluation
- Final drug product testing as the drug product manufacturing process can impact these attributes
- For Prefilled syringes
  - Enhanced washing procedures for prefilled syringes to reduce/remove tungsten
  - Tungsten specification
  - Consideration of different metal for needle hub on syringe (eg platinum)

# Product and Process Related Impurities Bearing on Immunogenicity



- Product-related impurities
  - Product variants, isoforms
  - Hydrolyzed/cleaved products
  - Intermediate products
  - Components of conjugated products
  - Misfolded or aggregated proteins
- ***Process-related impurities***
  - Host-cell components (e.g. HCP)
  - Remnants of adventitious agents
  - Selection agents, buffers
  - Leachates

# MAPP 5017.2

Establishing Impurity Acceptance  
Criteria as Part of Specifications for  
NDAs, ANDAs, and BLAs Based on  
Clinical Relevance

CONSIDERATION AND  
APPROACHES FOR BLAs



# Low levels of Innate Immune Response Modulating Impurities can Profoundly Impact Adaptive Immune Response

(Verthelyi D et al PLoS One. 2010;5(12); and PLoS One 2015;10(4))



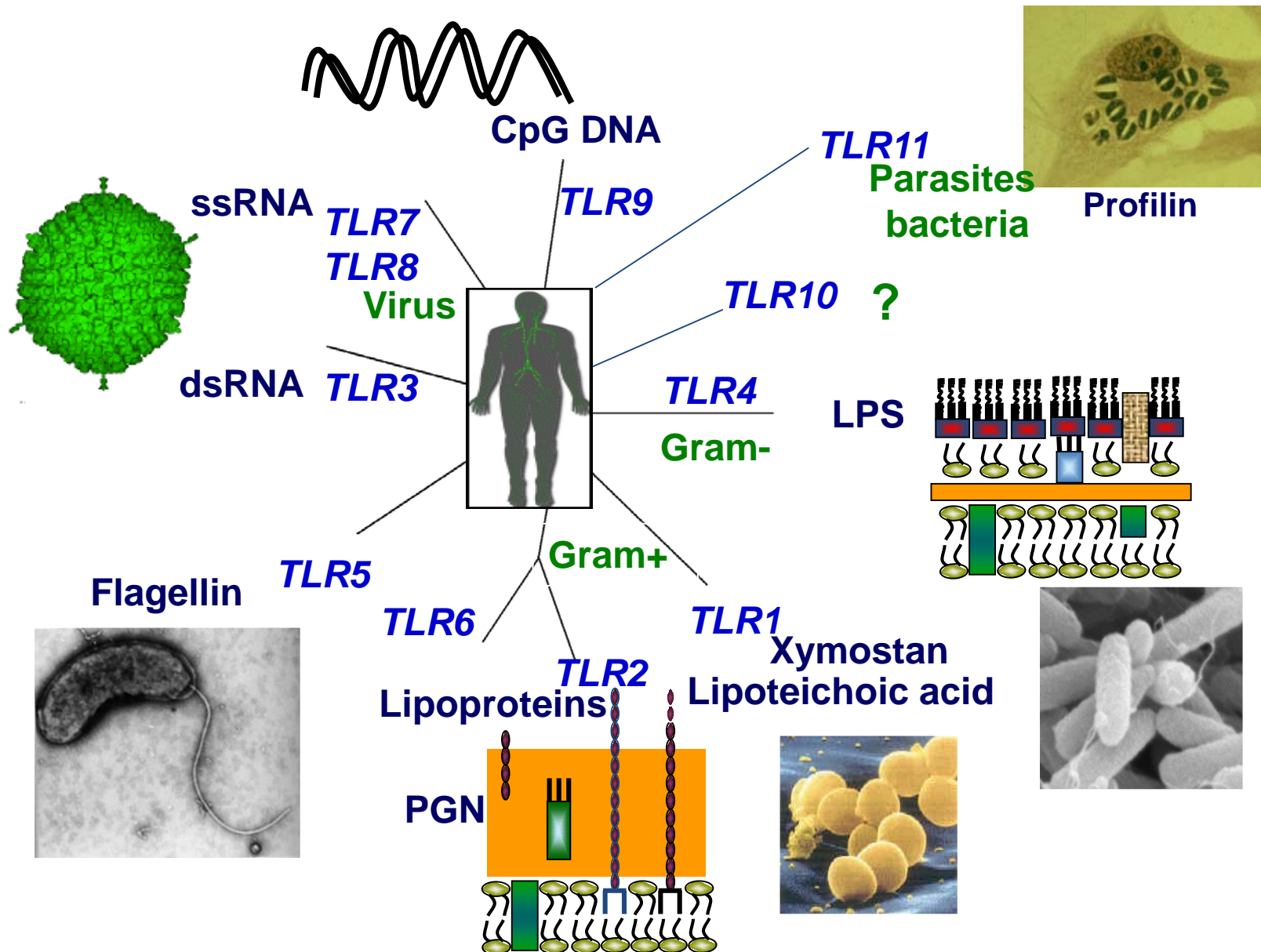
- Innate immune response modulating impurities (IIRMI)s activate local immune response at drug delivery/depot site
  - Induce an antigen-specific immune response to exogenous proteins.
  - Help break tolerance to endogenous proteins.
  - Change the quality of the response (IgG/isotype, affinity maturation)

# Innate Immune System: Detects Microbial Molecular Patterns, Stress and Tissue Damage



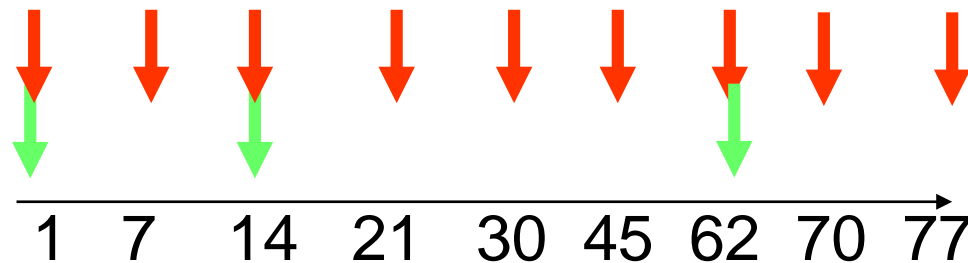
- Pathogen Associated Molecular Pattern (PAMP) Receptors
  - Toll like receptors (TLR)
  - C-Lectin Receptors (CLRs)
  - NLR (e.g. NOD1 & NOD2)
  - RNA Helicases (RIG-I, LGP2, & MDA-5)
  - Cytoplasmic receptors (e.g. DAI, IFI16, AIM2)
  - Scavenger Receptors: eg MARCO for particles
- Characteristics of PAMP Receptors
  - Recognize conserved molecular patterns in pathogens, damaged cells and stressed cells (DAMPs)
  - Exert action through common activation pathways
  - Genome encoded
  - Conserved throughout evolution
  - Formation of inflammasomes and generation of inflammatory response

# TLR : pattern-recognition receptors



# Can Low Levels of IIRMs Break Tolerance to a Self Protein?

(Verthelyi D et al 2010)



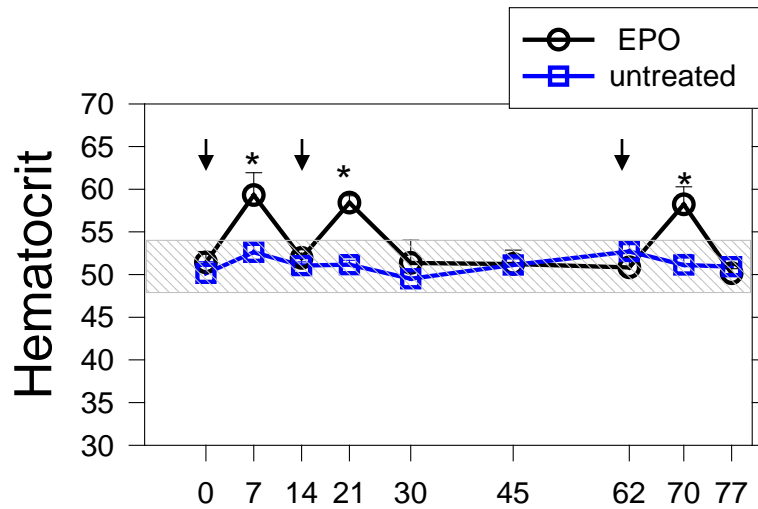
5 Balb/c mice/group

rhuEPO 10 ug/mouse SQ on days 1, 14 & 62

+/- CpGs or LPS

Hematocrit weekly

Human-mouse Epo homology= 80%



**Low levels of TLR agonists synergize to induce antibody response to break tolerance *in vivo***

# Control Strategy: Innate Immune Response Modulating Impurities



IIRMIIs that arise from fermentation (HCP, DNA)

- Validate removal by manufacturing process
- In process testing/specification
- Drug substance testing/specification

*IIRMIIs that arise from the manufacturing process* (e.g. endotoxin, leachates): recommendations for testing to inform immunogenicity risk

- In process testing/specification
- Drug substance and drug product testing/specification
- Caveats:
  - drug product generally too dilute for accurate measurement;
  - formulation may interfere with assays

- Haile et al. PLoS One, 2015 & J Pharm Sci. 106, 2017

# CQAs that Bear on Immunogenicity

- Protein origin: foreign vs self
- *Abundance of endogenous protein counterpart of therapeutic: degree of self-tolerance*
- Protein structure:
  - primary structure: sequence divergence or polymorphisms
  - aggregates
  - post-translational modifications/chemical degradation
- Impurities: process and product related
- Immunomodulatory properties of the protein therapeutic: immunostimulatory vs immune suppressive

# Low Abundance Self Proteins: Break in Tolerance to Thrombopoietin



- Levels of TPO in healthy volunteers in  $10^{-12}$  M range
- N-terminus contains receptor binding domain; C-terminus contains three MHC class II binding motifs and one promiscuous immunodominant T cell epitope (immunoinformatics analysis per Epivax).
- In preclinical tox studies, administration of *human* full length TPO to non-immune suppressed animals induced neutralizing antibodies (NABs) and thrombocytopenia: *attributed solely to contribution of xenogeneic determinants but....*
- Antibodies to full length TPO developed in 7% of treated *immune suppressed* cancer patients; few developed NABs, none developed thrombocytopenia.
- Administration of *species specific* full length TPO to non-immune suppressed monkeys and mice induced NABS and thrombocytopenia (G. Koren, Devel Biol 2002)
- Antibody response in animals developed initially to C-terminal and by epitope spread, then to the N-terminal receptor binding domain.



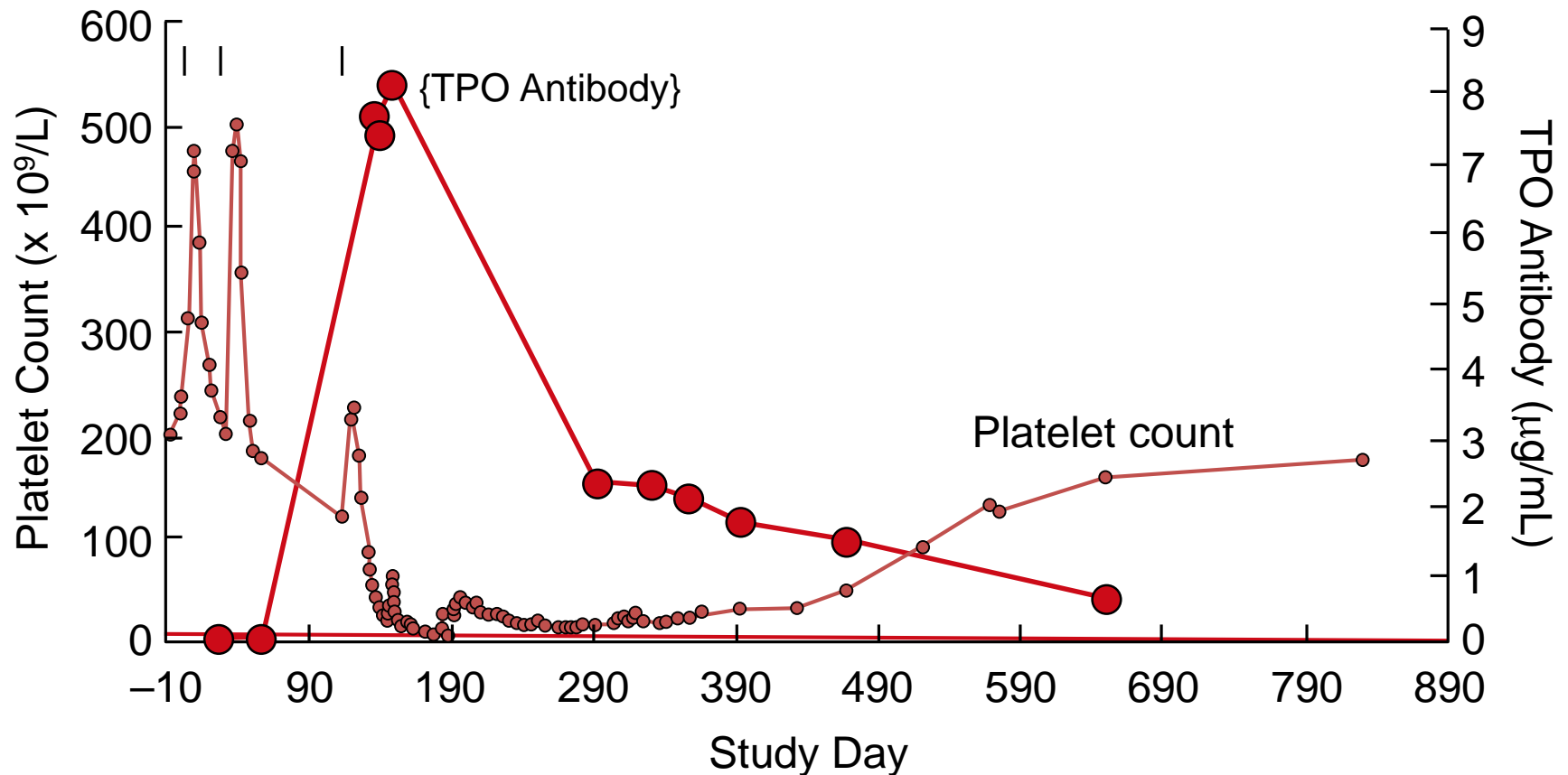
# Control Strategy for TPO Immunogenicity: Protein Engineering



- C-terminal truncation and pegylation
  - Elimination of highly immunogenic and non-essential C-terminus
  - Pegylation used to reduce immunogenicity and increase  $t^{1/2}$  of therapeutic proteins: “shielding” epitopes or altering antigen processing and presentation
- Neutralizing antibody to PEG-MGDF caused thrombocytopenia in healthy platelet donors: 13/325 (4%) and in immune suppressed oncology patients (0.5%) (Li et al 2001)
  - In some healthy donors, tolerance was easily broken (2-3 doses); argues against epitope spread, even from initial PEG or N-terminal domain site, as mechanism
  - Common HLA alleles in two formerly healthy patients: HLA-DQB5 0302/7, HLA-DR B1 04 and HLA-DR B4 01. Analysis to identify both high binding epitopes and corresponding class II alleles might further elucidate immune pathogenesis.

# rhuMGDF-PEG Breaks Tolerance in Healthy Volunteers

(Li et al 2001)



# Control Strategy 2 for TPO Immunogenicity: Development of TPO Mimetic



- Romiplostim, a member of the TPO *mimetic* class, is an Fc-peptide fusion protein (peptibody) that activates intracellular transcriptional pathways leading to increased platelet production via the TPO receptor (also known as cMpl).
- The peptibody molecule contains two identical single-chain subunits, each consisting of human immunoglobulin IgG1 Fc domain, covalently linked at the C-terminus to a peptide containing two thrombopoietin receptor-binding domains. *Romiplostim has no amino acid sequence homology to endogenous TPO.*

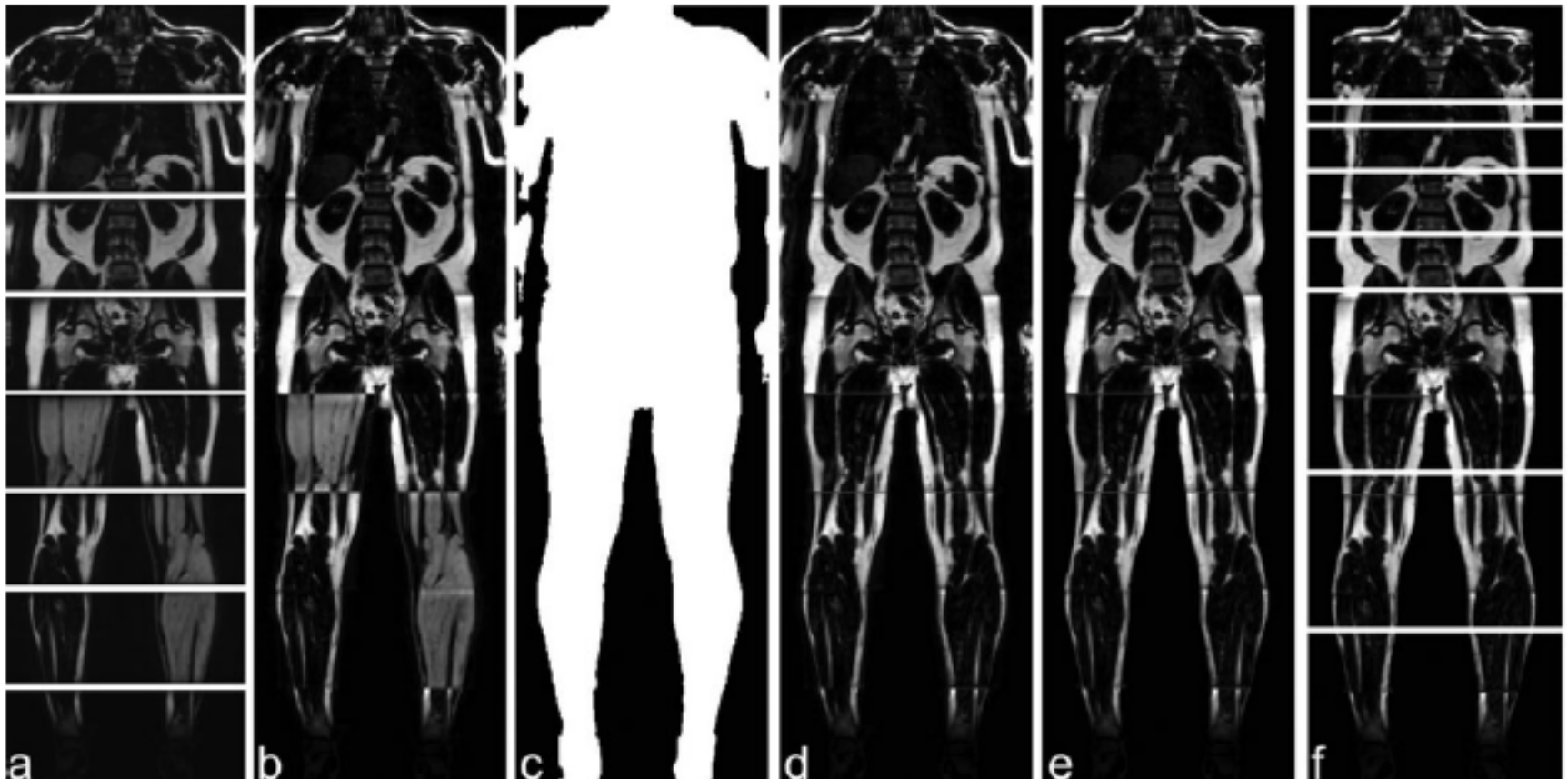
# Integrity of Protein Therapeutics in Storage Vessels Easily Monitored, Well Preserved



# Integrity of Therapeutic Proteins in in Vivo Environments Presents Challenges



(from Wald D et al 2012)

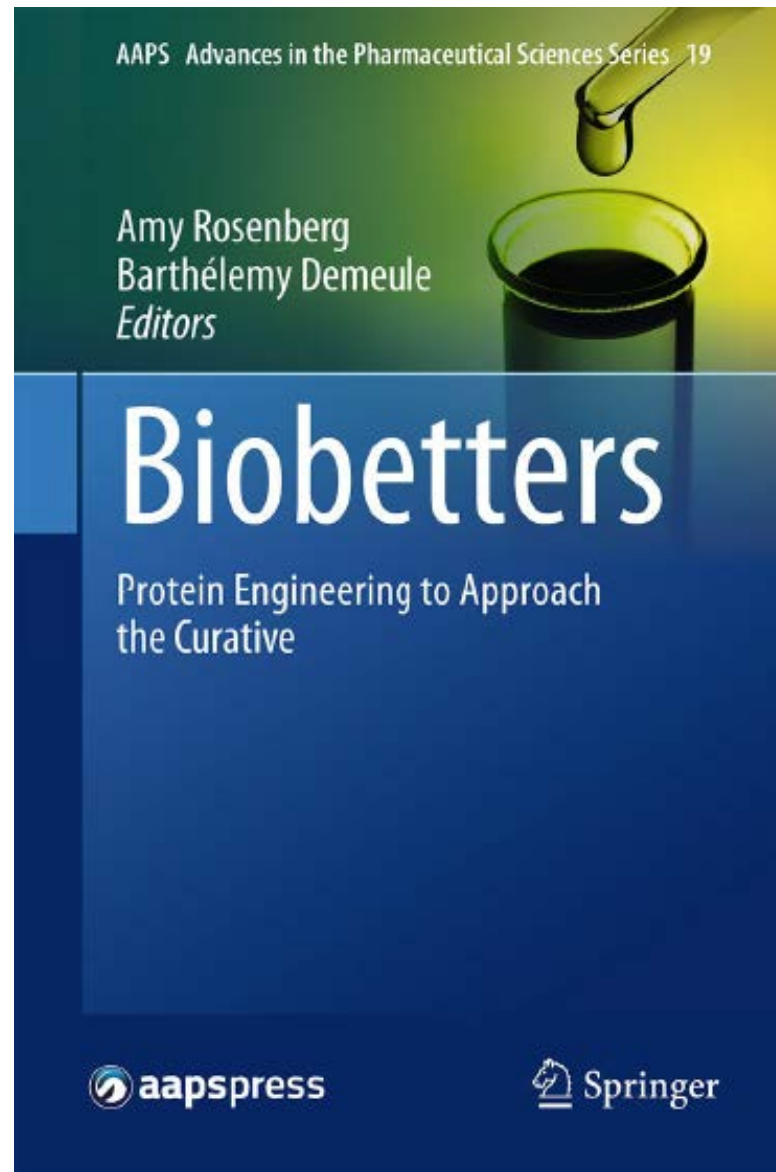


# Quality Target Product Profile Associated with Favorable Clinical Outcome but not Necessarily OPTIMAL Clinical Outcome

**ICH Q8(R2) QTPP Definition:** A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy.

**QTPP:** quality characteristics to ensure safety and efficacy as promised in the label

# Engineering of Protein Therapeutics for *Optimization* of Safety and Efficacy



# **“Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products”**

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)

August 2014  
Clinical/Medical



# Guidance for Industry

## Assay Development for Immunogenicity Testing of Therapeutic Proteins

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)**

December 2009  
CMC

# Summary

- Clinical relevance and control strategies to optimize quality target product profile, including clinically relevant specifications, are central to minimizing immunogenicity of therapeutic proteins and helping OPQ to meet patient expectations for quality
- Increased product and process knowledge can reduce residual immunogenicity risks and result in the development of more clinically relevant control strategies

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