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Analytical Tools for Higher Order Structure Assessment in Comparability and Biosimilarity

Brad Jordan, PhD
Director, Global Regulatory and R&D Policy
Amgen Inc.

CASSS HOS 2018

Providence, RI

How good are analytical methods?

Worthless copy or something more valuable?

Original Mona Lisa

Mona Lisa Del Prado

1. http://www.nytimes.com/2012/04/14/world/europe/prado-researcher-finds-insights-beneath-copy-of-mona-lisa.html
2. http://www.nytimes.com/interactive/2012/04/14/world/europe/Not-Just-Another-Fake-Mona-Lisa.html

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Ana Gonzalez Mozo

Expert Art Curator

Mona Lisa Del Prado

1. http://www.nytimes.com/2012/04/14/world/europe/prado-researcher-finds-insights-beneath-copy-of-mona-lisa.html
2. http://www.nytimes.com/interactive/2012/04/14/world/europe/Not-Just-Another-Fake-Mona-Lisa.html

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How good are analytical methods?

Mona Lisa Del Prado is the oldest known replica

Painted alongside Da Vinci in his studio as he painted the original

1. http://www.nytimes.com/2012/04/14/world/europe/prado-researcher-finds-insights-beneath-copy-of-mona-lisa.html
2. http://www.nytimes.com/interactive/2012/04/14/world/europe/Not-Just-Another-Fake-Mona-Lisa.html

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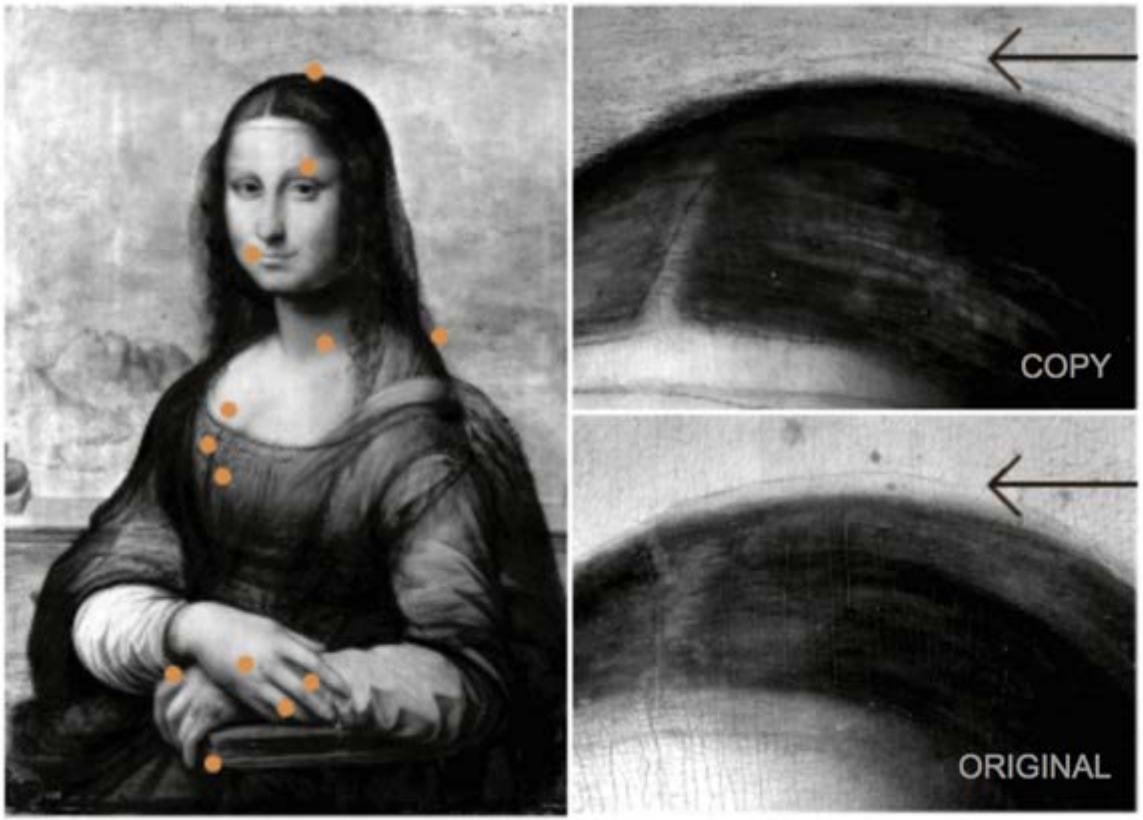
How good are analytical methods?



The Discovery
Infrared reflectography reveals drawing lines under the paint, invisible to the naked eye. Every adjustment that Leonardo made on his underlying drawing was repeated in the copy, indicating that the two pieces were painted in tandem. The orange dots show where adjustments were made in both paintings. The arrows point to adjustments made to the head by the two painters.

http://www.nytimes.com/2012/04/14/world/europe/prado-researcher-finds-insights-beneath-copy-of-mona-lisa.html

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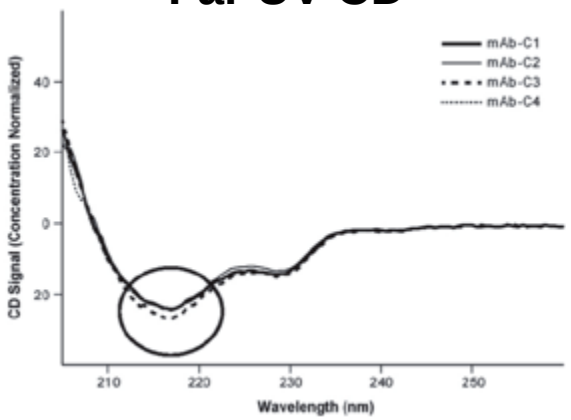
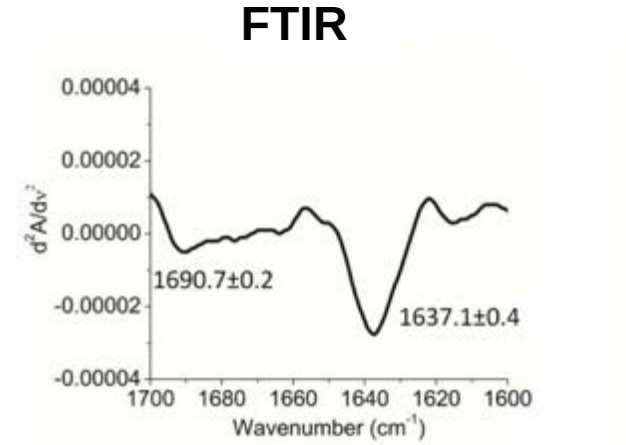
Current state: What analytical data can and can't tell us

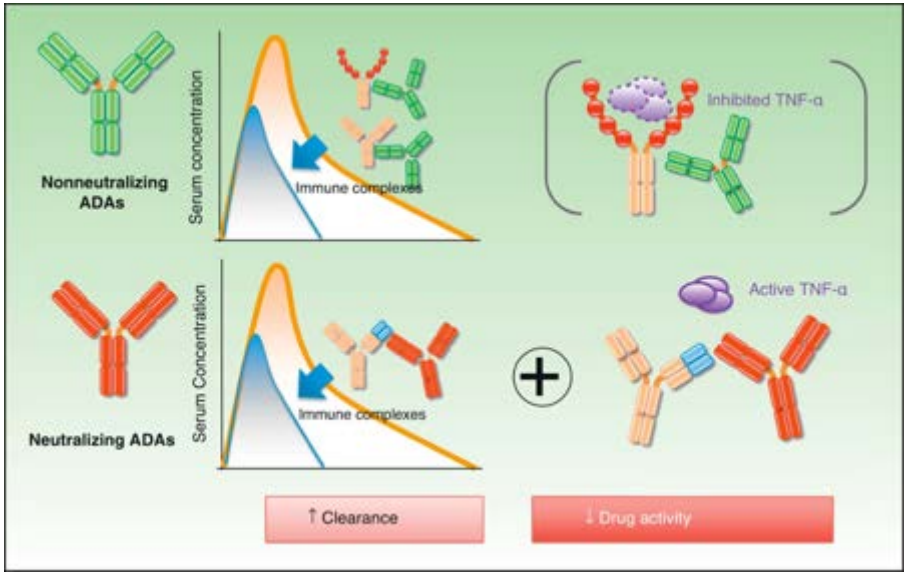
Far UV CD



FTIR



But they cannot predict safety/efficacy?



1. Chen et al. Reconstruction of 3D structures of MET antibodies from electron microscopy 2D class averages, PLOS one, 2017, 12(4).
2. Carrascosa, JM. Immunogenicity in Biologic Therapy, Implications for Dermatology, ACTA Dermo-Sifiiograficas, 2013, 104, 471-479.
3. Srivalli, N. et al. Structural Characterization of IgG1 mAb Aggregates and Particles Generated under various Stress Conditions, *J Pharm Sci*, 2014, 103(3), 796-809.

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Reliance on prior knowledge: supportive data

Process Comparability
Batch Analysis
Product Characterization
Integrated Control Strategy

Can rely on manufacturer product/process understanding, control strategies, and a risk-based approach to comparability design

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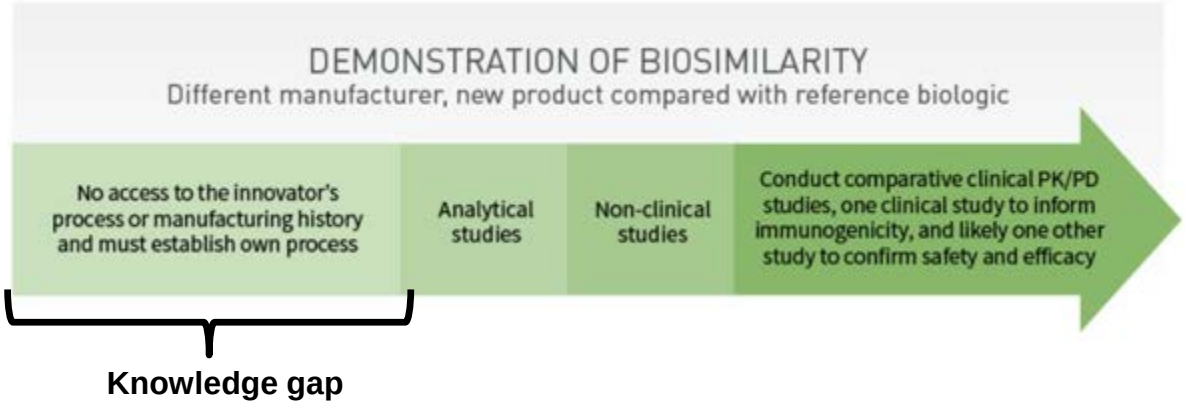
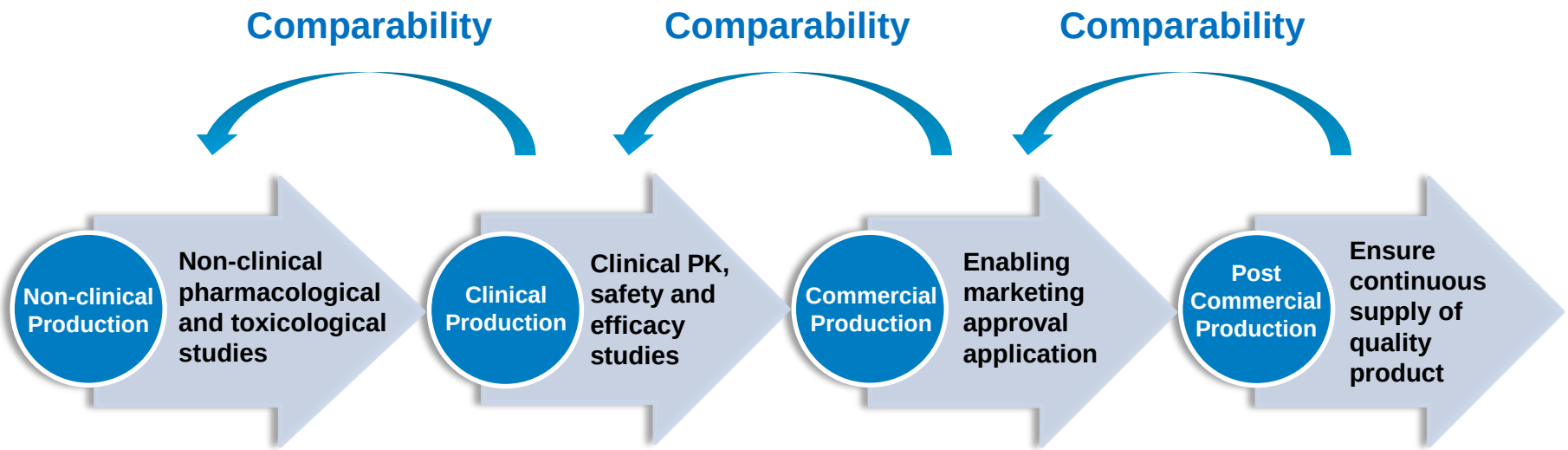
Biosimilar manufacturers will not have access to the knowledge base possessed by the reference product manufacturer and must design and implement their own control strategies



1. http://www.amgenbiosimilars.com/manufacturing/comparability-vs-biosimilarity/

Comparability compares pre- and post-change product taking into account prior knowledge



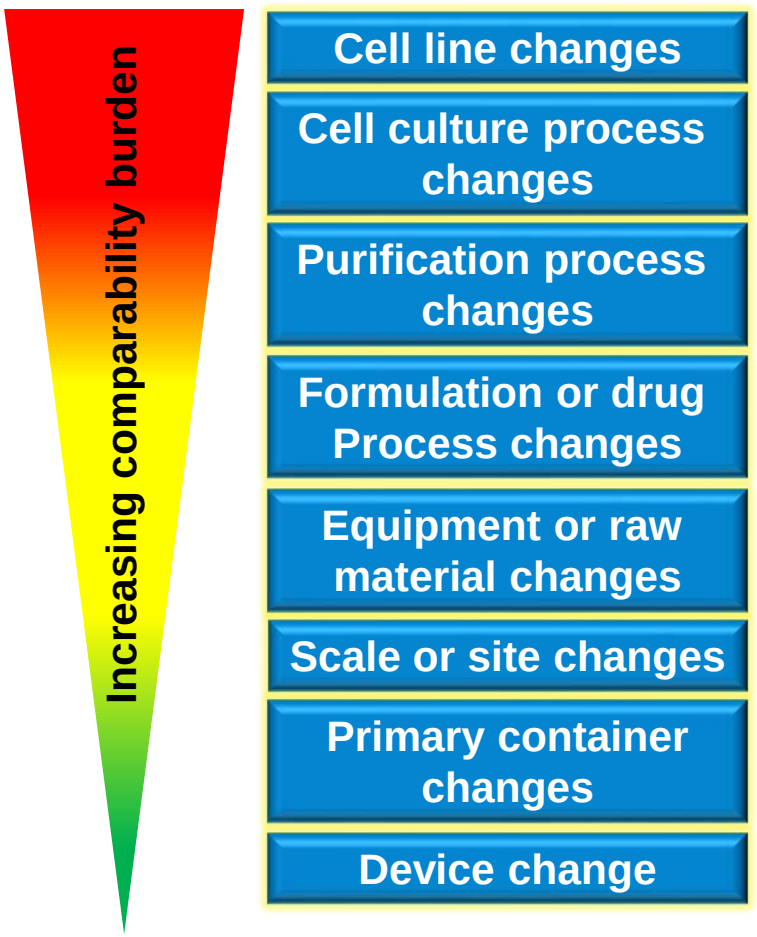
Comparability is a stand-alone event after approval

Manufacturers understand product attributes and implement appropriate control strategies

In many cases supportive data may be enough

Comparability uses risk-based approaches based on type and extent of process changes

Understanding of product and process allows risk-assessment



Clinical safety and efficacy

- Confirmation of comparable efficacy, safety, and immunogenicity
- Post change surveillance

Nonclinical or clinical PK-PD

- Animal studies (rodent or primates)
- Human studies

Biologically comparable

- Potency and target binding
- Additional biological activities such as effector functions
- Other known mechanism of action of the product

Analytically comparable

- Physicochemical properties including higher order structure (HOS)
- Stability profiles
- Impurities and contaminants

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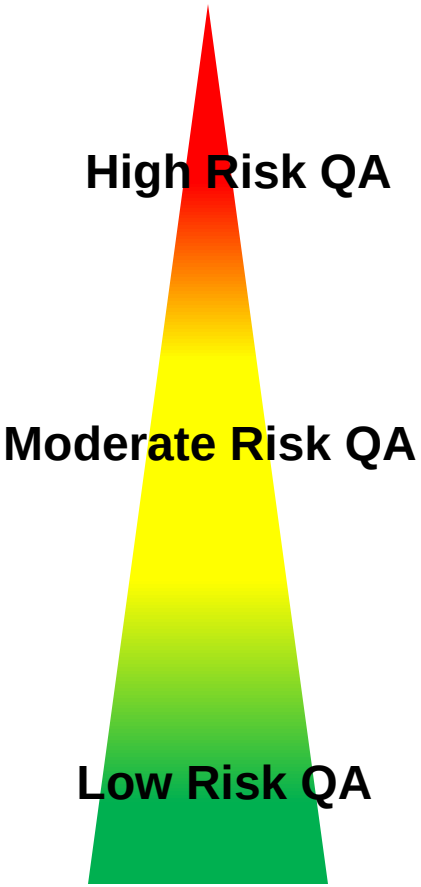
Similarity assessment plan is based on risk ranking of *reference product* attributes



High Risk Quality Attributes

- Ranges defined by reference product; May be narrowed based on assay variability; Statistical approach should be justified
- Known or probable interactions in product performance may limit the ranges of certain attribute combinations
  - The use of one attribute to compensate for a difference in another may be challenging as the attributes may have other effects

Moderate Risk Quality Attributes

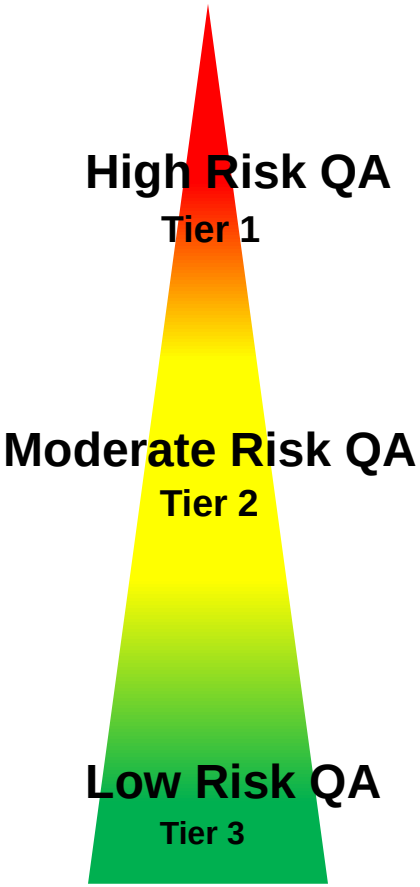
- Ranges defined by reference product ranges or other appropriate scientific justification
- Known or probable interactions that have a higher risk of impacting product performance should be treated as a higher risk attribute

Low Risk Quality Attributes

- May not have target ranges
- Known or probable interactions that have a higher risk of impacting product performance should be treated as a higher risk attribute
- Even though these attributes may not need to match the reference product, the closer the overall match the greater the confidence that unmeasured attributes are not different

1. Slide concept from Kozlowski, S (CDER) presentation at CASSS WCBP, Washington DC, January 31, 2013

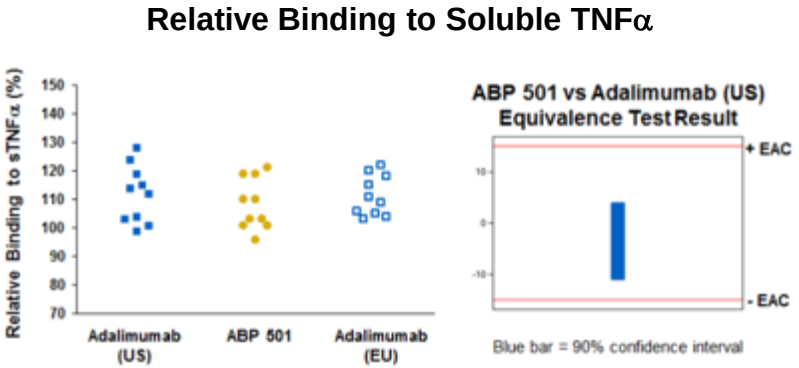
Similarity uses tiered approach for risk ranking and statistical analysis of quality attributes

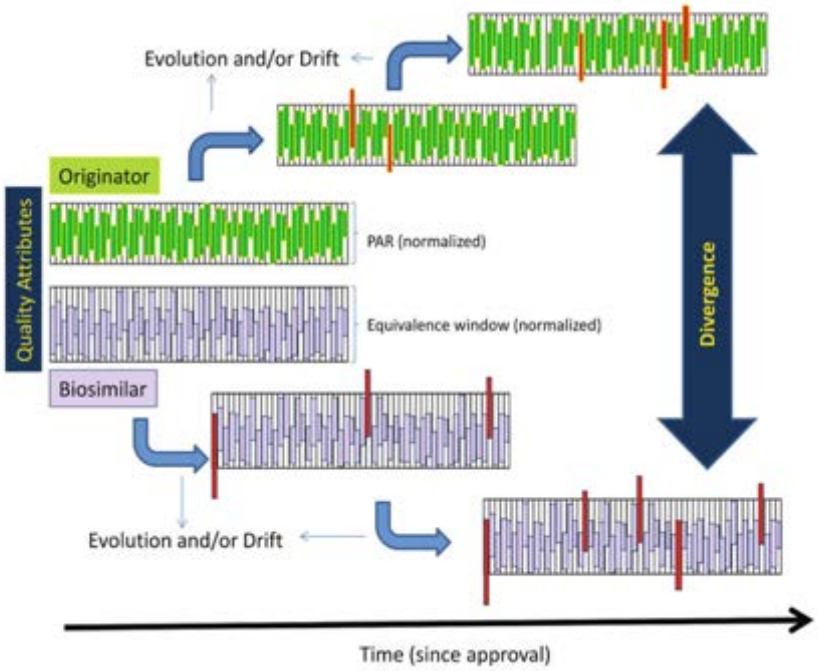


Equivalence
$(\pm 1.5\,\sigma)$

Quality Range

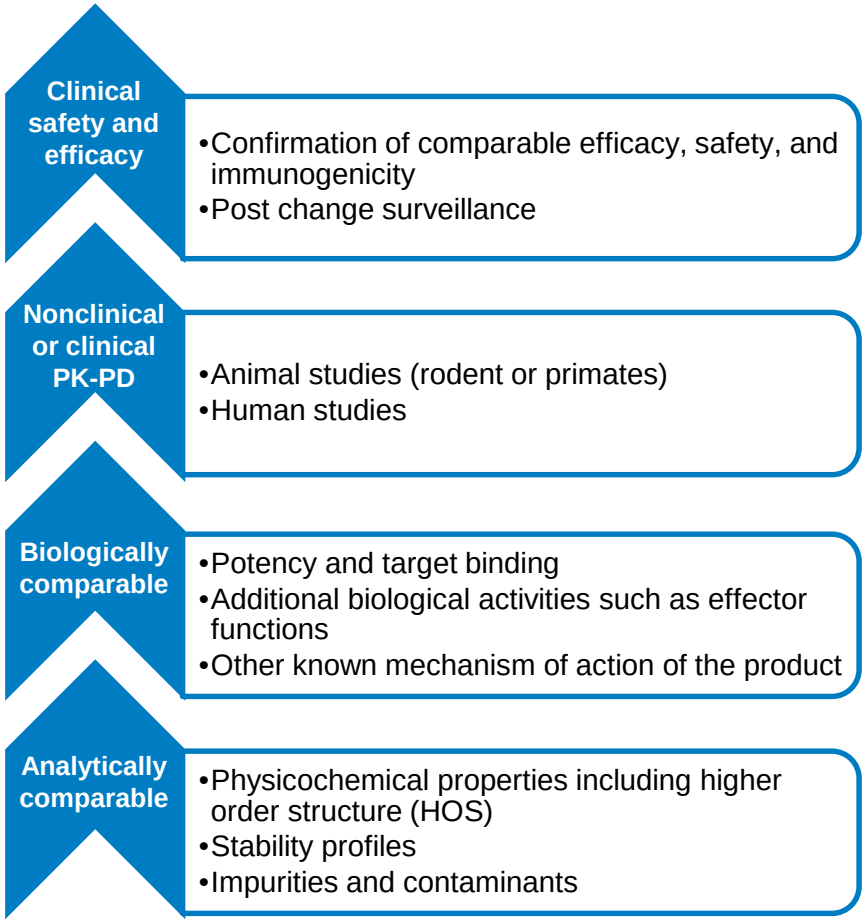
Visual Inspection





1. https://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/arthritisadvisorycommittee/ucm510293.pdf
2. Ramanan, S. and Grampp, G., Drift, Evolution, and Divergence in Biologics and Biosimilars Manufacturing, BioDrugs, 2014, 28, 363-372.

Analytics can establish high similarity but even current techniques leave ‘residual uncertainty’



Clinical studies in a sensitive patient population can address residual uncertainty

ICH Q5E

Although the pre- and post change product appear highly similar, the analytical procedures used are not sufficient to discern relevant differences that can impact the safety and efficacy of the product. The manufacturer should consider employing additional testing (e.g., further characterisation) or nonclinical and/or clinical studies to reach a definitive conclusion;

1. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5E/Step4/Q5E_Guideline.pdf

The Value of Clinical Data
Case Study: Epoetin alfa process change

Comparability protocol after complex changes to cell culture

Structural and Functional Attribute	Comparability Finding
Primary Structure (peptide mapping and sequencing)	Comparable AA seq and S-S linkage
Higher Order Structure (CD, FT-IR)	Comparable HOS
Carbohydrate Profile (sialo and asialo N-glycan mapping, peptide map, isoform distribution)	*Similar* qualitative profile – within specs • Slightly higher acidic isoforms – but within spec • Slightly higher N-glycans with N-acetyllactosamine extensions • Reduced N-glyco neuraminic acid • Higher bisialylated O-glycans • Comparable N-glycan sialylation
Purity and Impurities	Comparable and within spec
In vitro bioassays	Comparable and within spec
Exhypoxic polycythemic mouse bioassay	Comparable potency

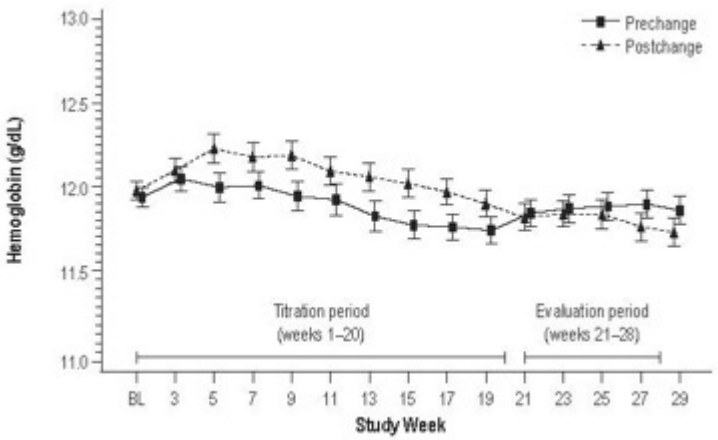
Clinical Bridging Study	Comparability Finding
30 patient, single-dose crossover study (16-20 week titration)	90% CI, 94.8%-104.3% - comparable PK

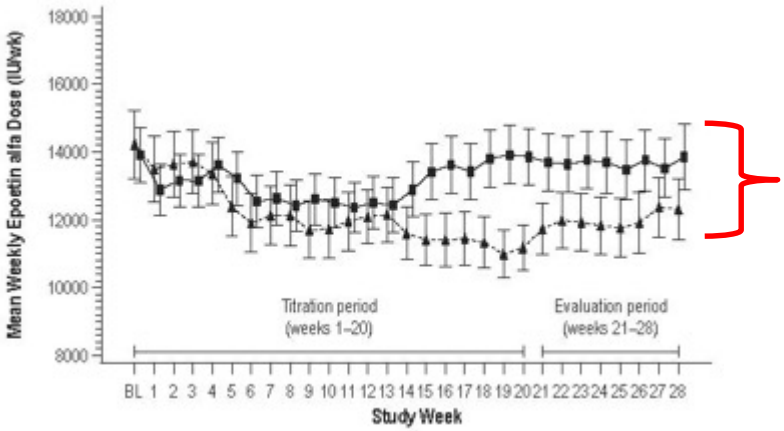
Analytically Comparable

1. Grampp, G. et al. Structure-Function Relationships for Recombinant Erythropoietins: A Case Study From a Proposed Manufacturing Change with Implications for Erythropoietin Biosimilar Study Designs, J. Pharm. Sci., 2018, epub ahead of print.

The value of clinical data
Case Study: Epoetin alfa process change

Clinical Evaluation: Hemoglobin Maintenance





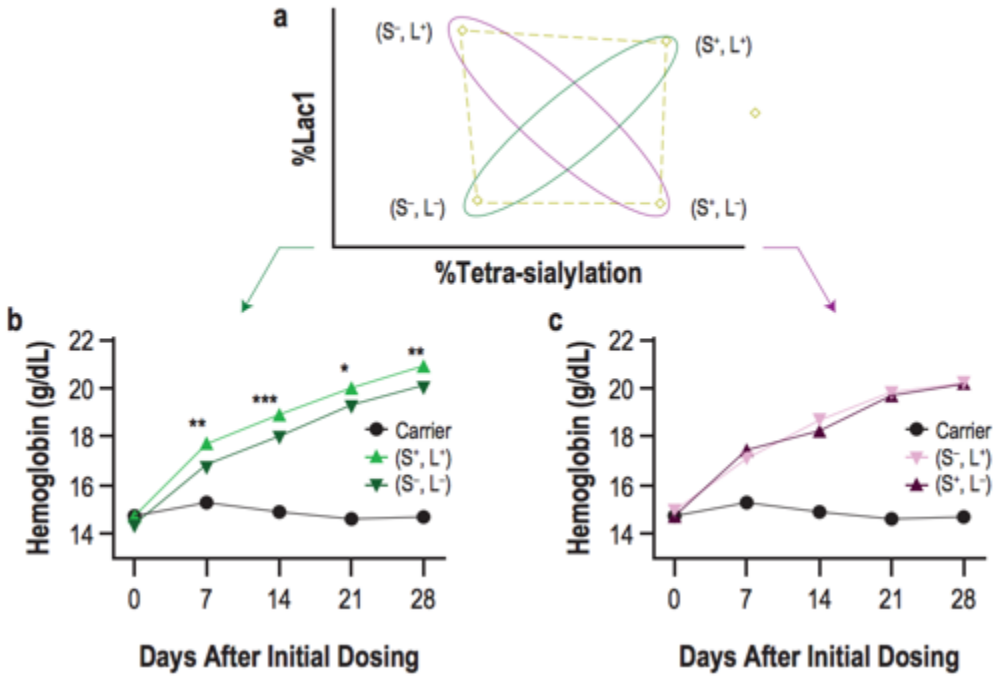
- **462 patient hemoglobin maintenance study**
- **Equivalence in hemoglobin levels demonstrated (Delta of -0.07 g/dL)**
- **Log dose ratio over the *last 8 weeks* of the study demonstrated the product from the new process to be more potent than pre-change product**

Analytics were not able to accurately predict this clinical observation

1. Grampp, G. et al. Structure-Function Relationships for Recombinant Erythropoietins: A Case Study From a Proposed Manufacturing Change with Implications for Erythropoietin Biosimilar Study Designs, *J Pharm Sci*, 2018, epub ahead of print.

The value of clinical data
Case Study: Epoetin alfa process change

In vitro potency assays can help understand differences



- Mouse hemoglobin accumulation model to assess *in vitro* effects
- Pairwise comparison of S^+,L^+ samples and S^-,L^- demonstrated statistically significant differences in hemoglobin levels due to additive impact of sialylation and lactosamine content
- Lactosamine content can offset effects of increased sialylation

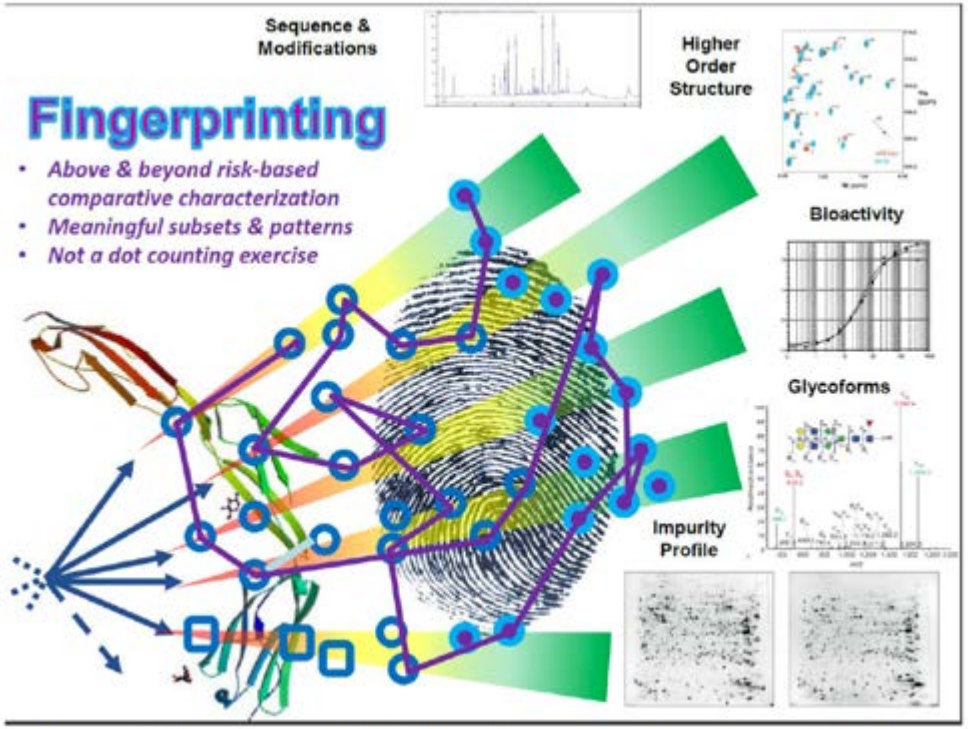
Orthogonal methods are needed to support analytical tools

1. Grampp, G. et al. Structure-Function Relationships for Recombinant Erythropoietins: A Case Study From a Proposed Manufacturing Change with Implications for Erythropoietin Biosimilar Study Designs, J. Pharm. Sci., 2018, epub ahead of print.

Could better analytics have predicted this?

FDA Definition of Fingerprint-like similarity:

"integrated, multi-parameter approaches that are extremely sensitive in identifying analytical differences."[1]

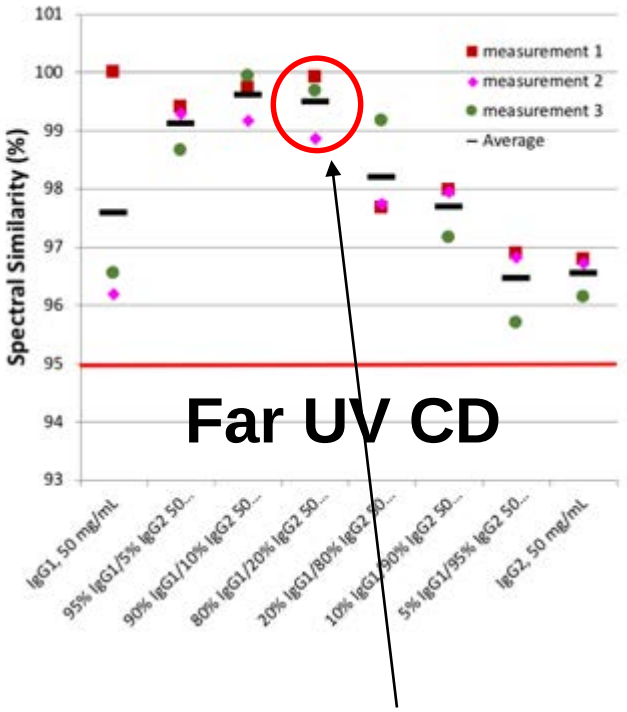


Koslowski, presentation at CASSS WCBP Conference, Washington DC, Jan, 31 2013

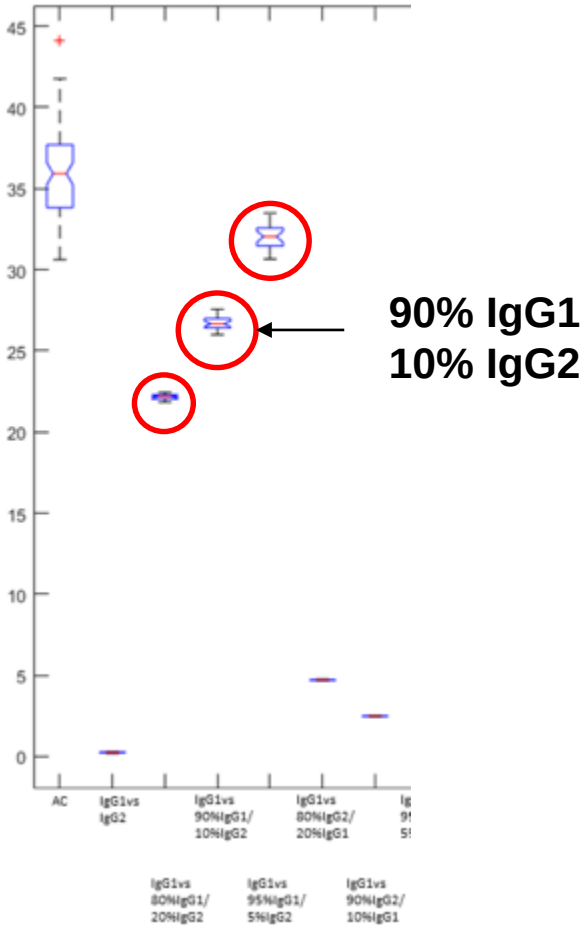
?

1. FDA Guidance, "Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product", https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm397017.pdf

More sensitive techniques allow detection of differences not previously observed



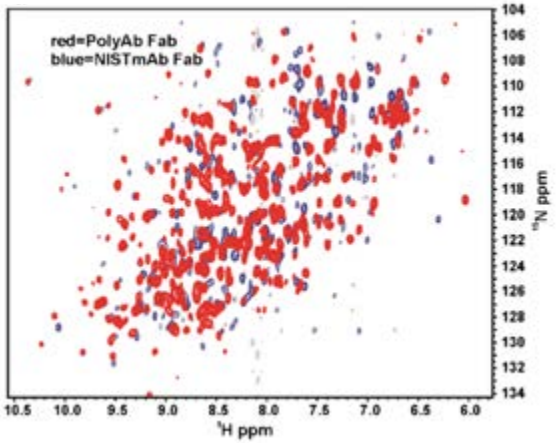
PROFILE NMR



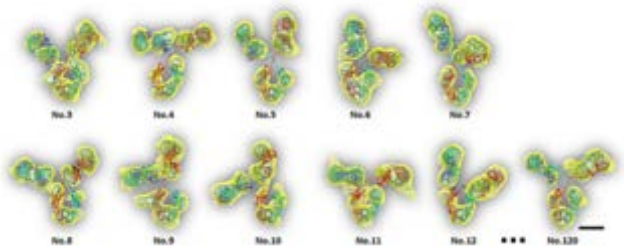
Mats Wikström, CASSS HOS 2018. "NMR represents a superior method for the assessment of higher order structure (HOS) of biopharmaceuticals.

Advancements in technology will allow lower detection limits and additional structural insights

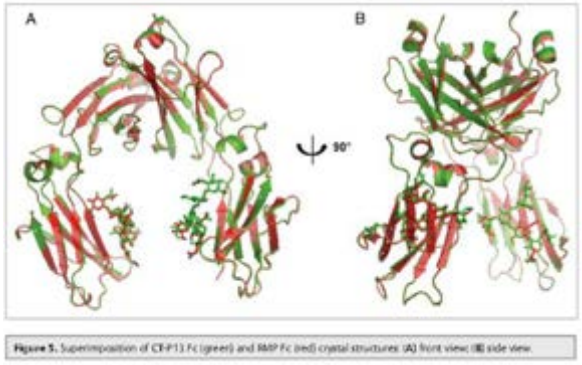
2D NMR Methods[1]



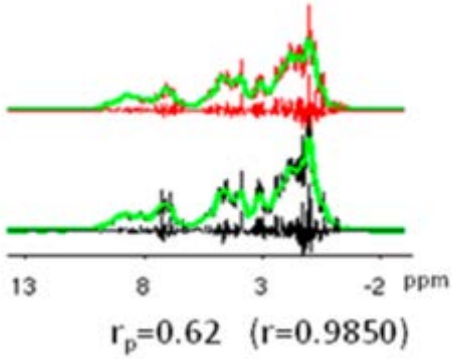
Cryo-EM[3]



X-ray Crystallography[2]



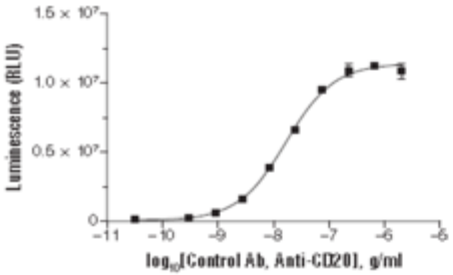
1D NMR PROFILE[4]



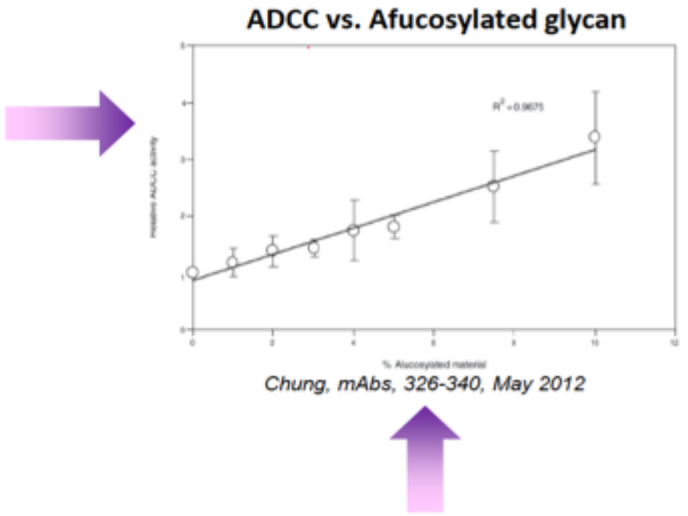
‘Fingerprint-Like’ HOS may reduce residual uncertainty, but some clinical data will likely be needed to inform safety and efficacy

1. Arbogast et al., Mapping Monoclonal Antibody Structure by 2D ^{13}C NMR at Natural Abundance, Anal. Chem., 2015, 87(7), 3556-3561.
2. Jung et al., Physicochemical characterization of Remsima®, mAbs, 2014, 6(5), 1163-1177.
3. Zhang et al. 3D Structural Fluctuation of IgG1 Antibody Revealed by Individual Particle Electron Tomography, Nature, 2015, 5, 9803.
4. Poppe et al., Profiling Formulated Monoclonal Antibodies by ^{1}H NMR Spectroscopy, Anal. Chem., 2013, 85(20), 9623-9629.

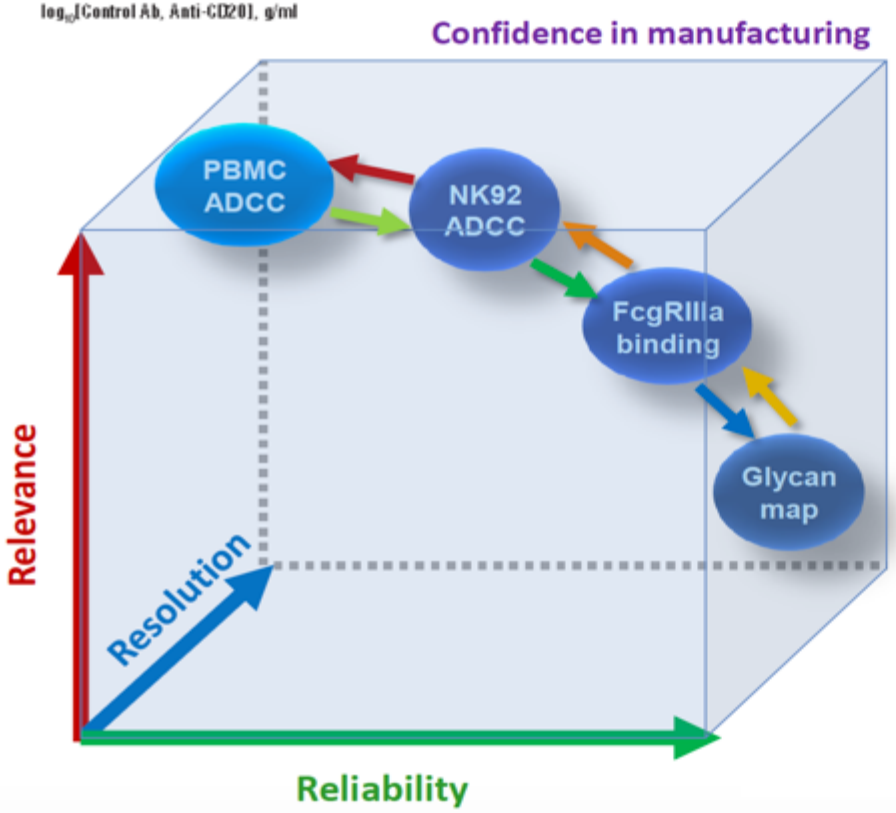
Deeper understanding of process and products will further reduce residual uncertainty



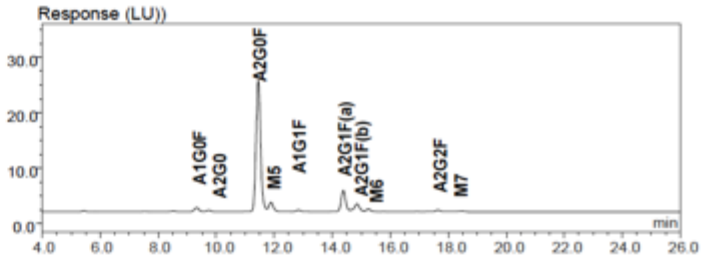
Orthogonal assays with increasing biological relevance to discern clinically meaningful differences and confirm functional similarity



Chung, mAbs, 326-340, May 2012



High resolution and reliable methods suited for process control can guide the process and product development.



Summary

- Analytical techniques for characterizing higher order structure have evolved and can assist in reducing residual uncertainty
- Analytical data are *not* a substitute for clinical data
- Fingerprint-like similarity, when a reality, may better inform the type and degree of clinical data needed
- Greater understanding of product, process, and control strategy reduces residual uncertainty

Acknowledgements

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