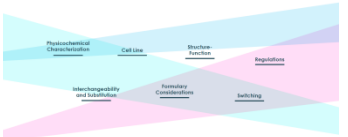
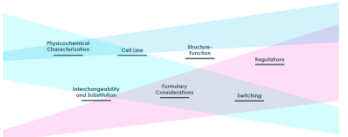


Brad Jordan, PhD, USA

- Director, Global Regulatory Policy, Amgen Inc, USA



20 November 2017, Holiday Inn Izdihar Riyadh, Saudi Arabia

Biologicals and biosimilars – totality of evidence

Brad Jordan, PhD

20 November 2017

Discussion Topics

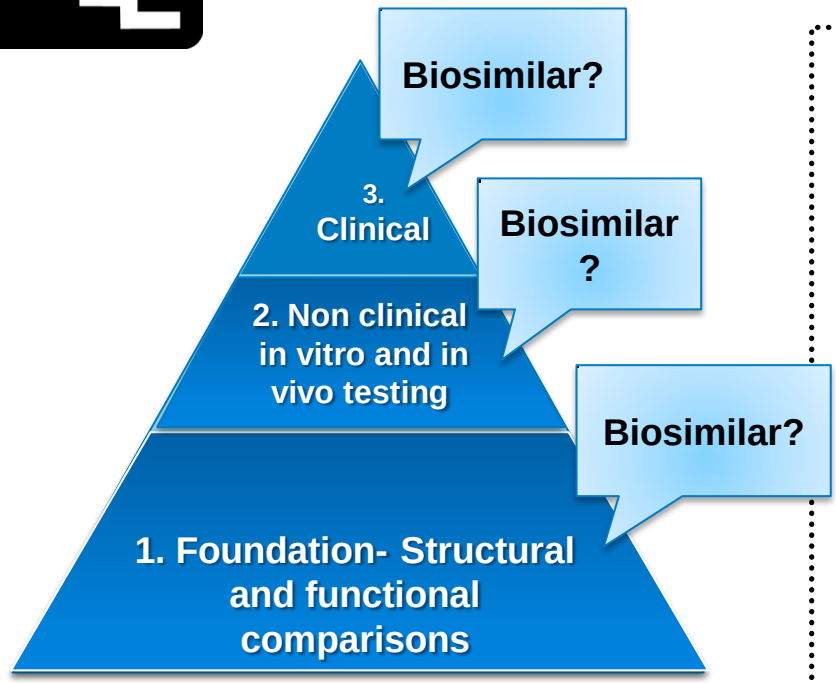
- Overview of biosimilar development
- Elements and limitations of analytical studies
- Role of structure-function studies

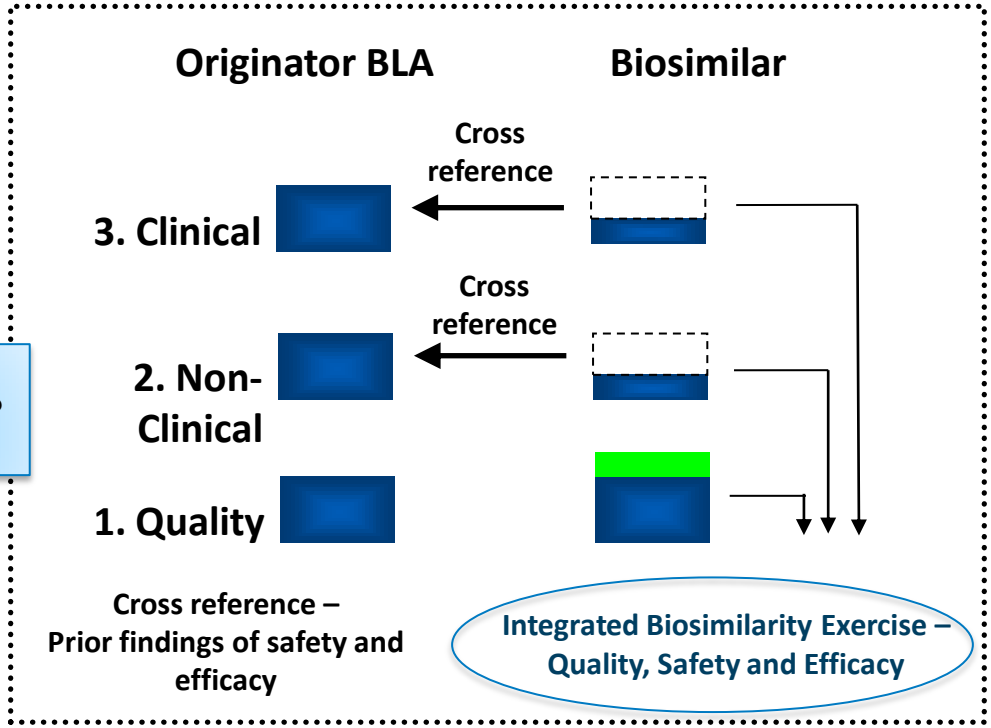
Discussion Topics

- Overview of biosimilar development
- Elements and limitations of analytical studies
- Role of structure-function studies

Biosimilar development proceeds through a stepwise similarity exercise

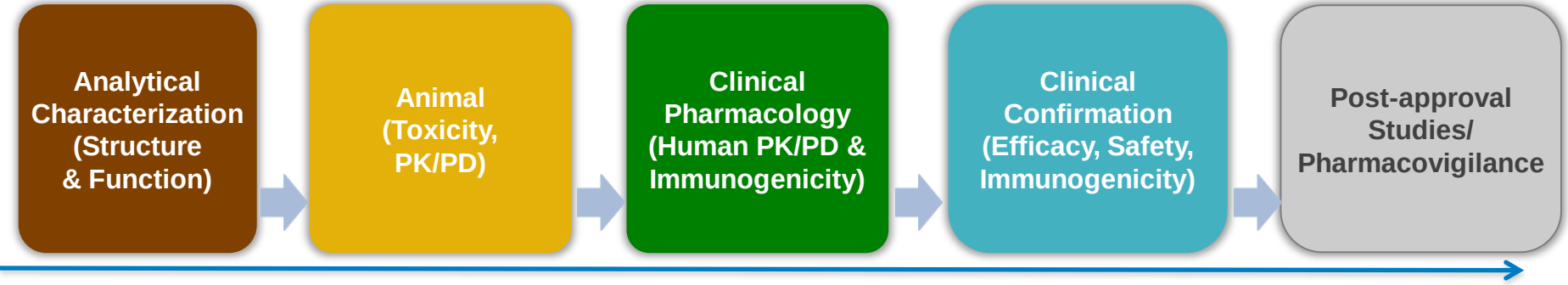
Approval is Based on the "Totality of Evidence"[1]





Food and Drug Administration. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf. Accessed 24 January 2013.

Biosimilar development and approval is based on the totality of evidence



- Each step uses scientific rigor and state-of-the-art capabilities
- Each step independently supports similarity and combined demonstrate a 'highly similar' product
- "Totality of evidence" approach is used for regulatory approvals

US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015.

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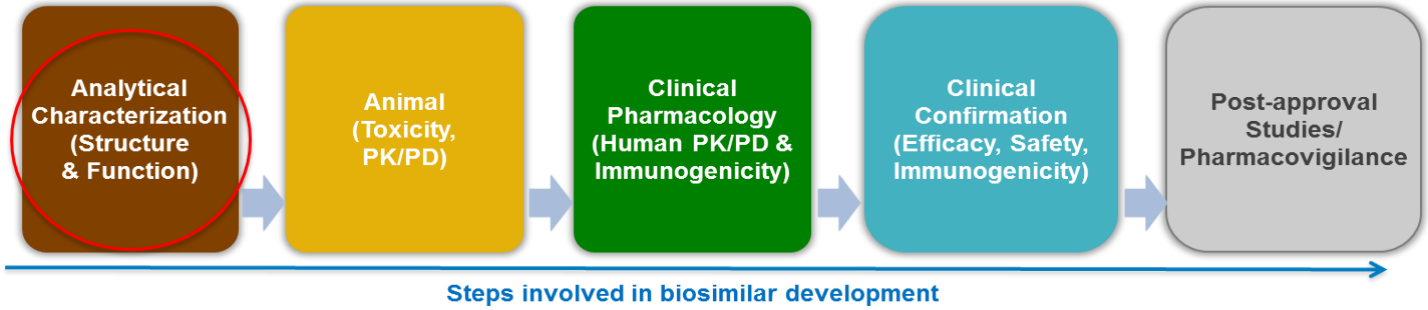
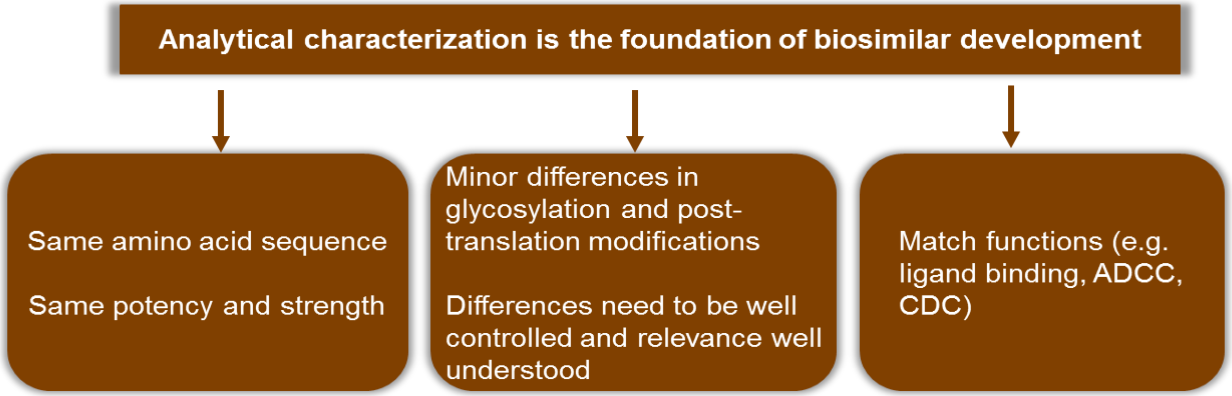
Analytical characterization is the foundation of biosimilarity demonstration





US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015.

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Analytical methods should be sensitive to assess protein structure and drug product characteristics

Amino acid sequence and modifications

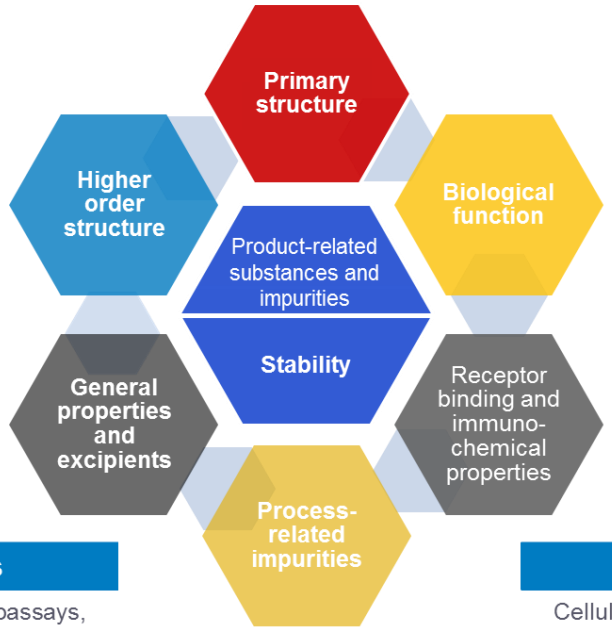
Molecular mass, peptide mapping with multiple proteases

Folding

S-S bonding, differential scanning calorimetry, circular dichroism, Fourier transform infrared spectroscopy

Subunit interactions

Analytical ultracentrifugation, field flow fractionation, light scatter, micro-flow Imaging



Glycosylation

Hydrophilic chromatography, enzymatic digestion, peptide mapping, CE, MS

Size, aggregates, charge, hydrophobicity

Size-exclusion chromatography, capillary gel electrophoresis, ionic-exchange chromatography, capillary isoelectric focusing

Impurities

Proteomics, immunoassays, real-time PCR analysis

Bioactivity

Cellular and *in vivo* bioassays, ligands and receptor binding (ELISA, surface plasmon resonance), signal transduction

CE, capillary electrophoresis; ELISA, enzyme-linked immunosorbent assay; MS, mass spectrometry; PCR, polymerase chain reaction; S-S, disulfide.

1. Shapiro M. Advisory Committee for Pharmaceutical Science and Clinical Pharmacology Meeting. August 8, 2012. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AdvisoryCommitteeforPharmaceuticalScienceandClinicalPharmacology/UCM315764.pdf Accessed March 2015.

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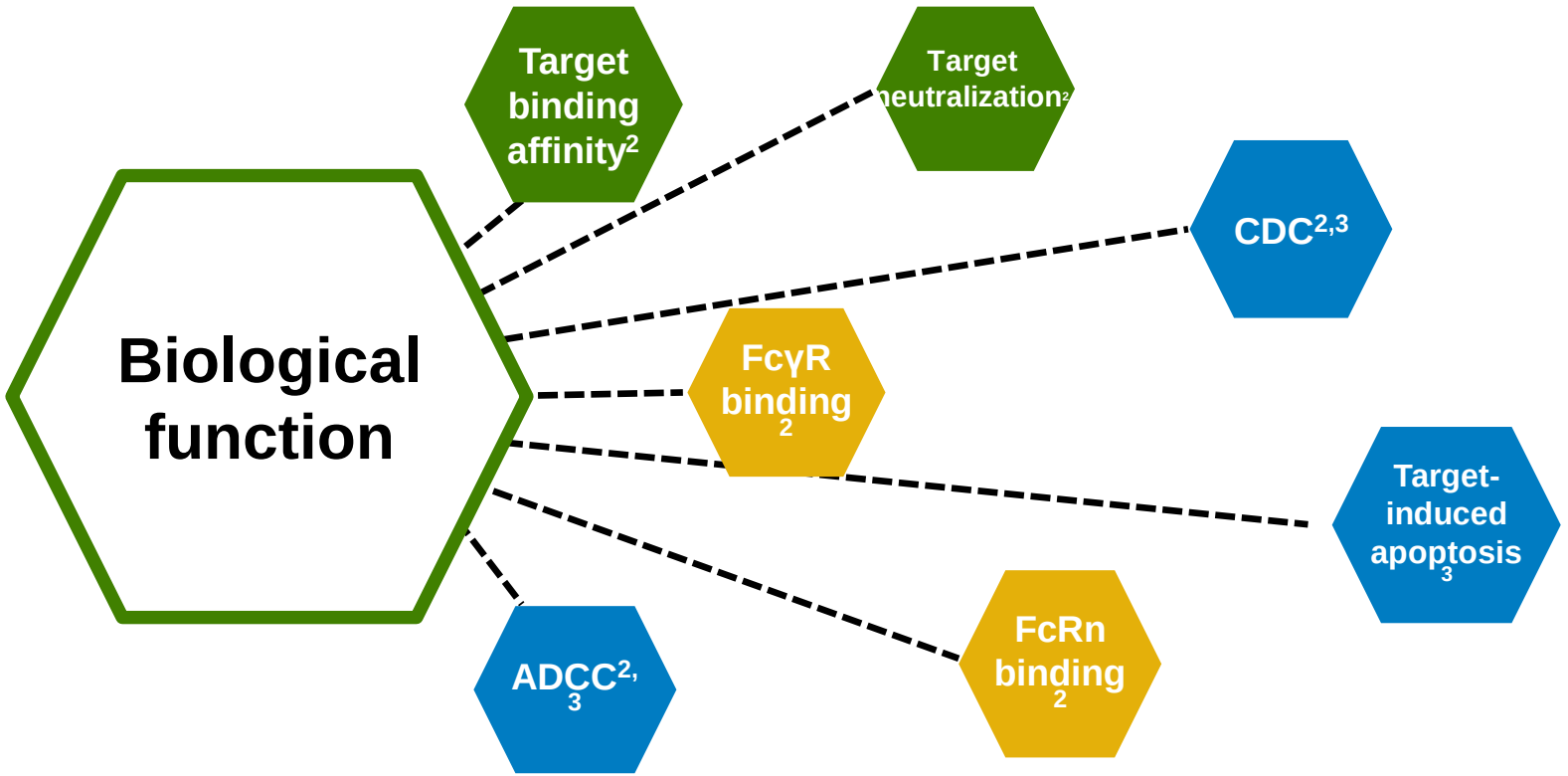
Biosimilars should match the activity (function) of the reference product[1]



1. US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015. 2. Reichert JM. *mAbs*2011;3:223–240.3. Peake STC *et al. Inflamm Bowel Dis* 2013;19:1546–1555.

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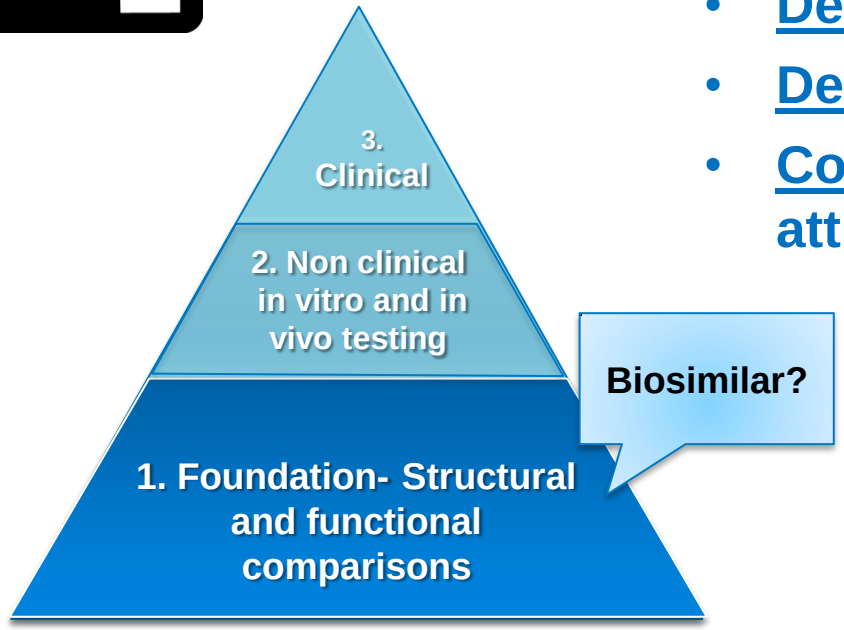
Process design and analytical studies form the foundation of biosimilar development

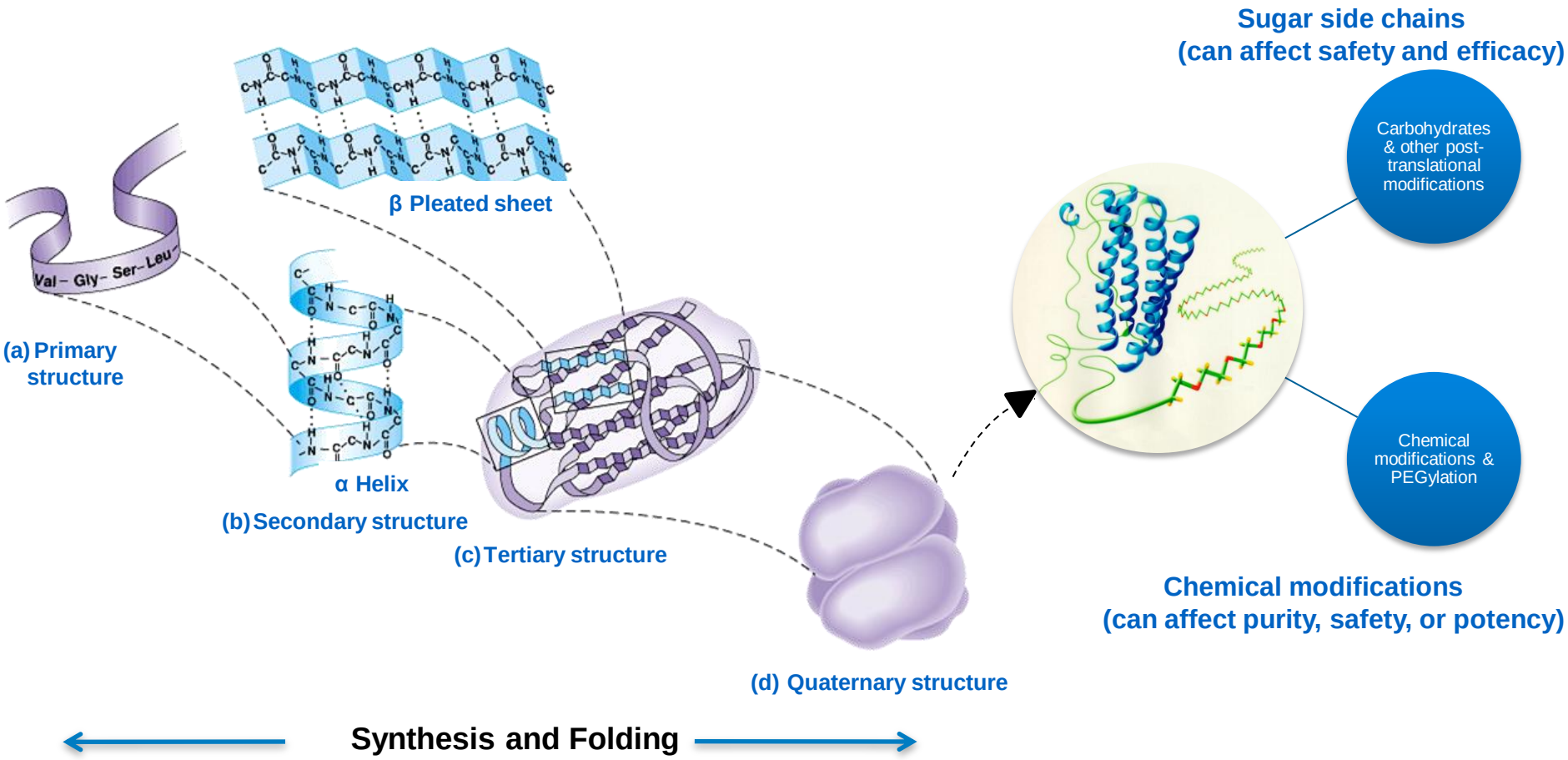


Before non-clinical and clinical testing can proceed:

- Define the target quality profile
- Design the process
- Compare structural and functional attributes

- **Use state-of-the-art analytical characterization and functional assays to assess any structural difference**
- **Understand the importance and limitation of functional assays**

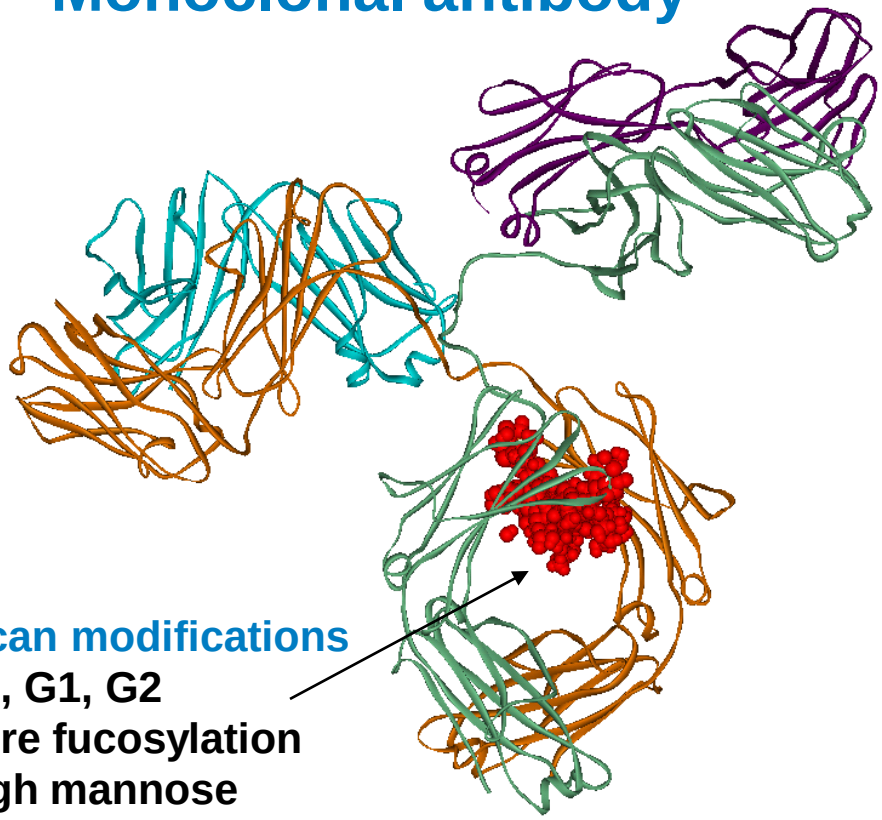
Biologics may have 4 orders of structure plus modifications that affect in vivo characteristics



[Image Source: Tim Osslund; Amgen Usage Rights: Unlimited world-wide usage rights for an unlimited time; http://kvhs.nbed.nb.ca/gallant/biology/protein_structure.html.
Data source: USP-NF 1045. Biotechnology-derived articles: 3-20]

Biological products have very complex structures

Monoclonal antibody



Glycan modifications
- G0, G1, G2
- Core fucosylation
- High mannose
- etc

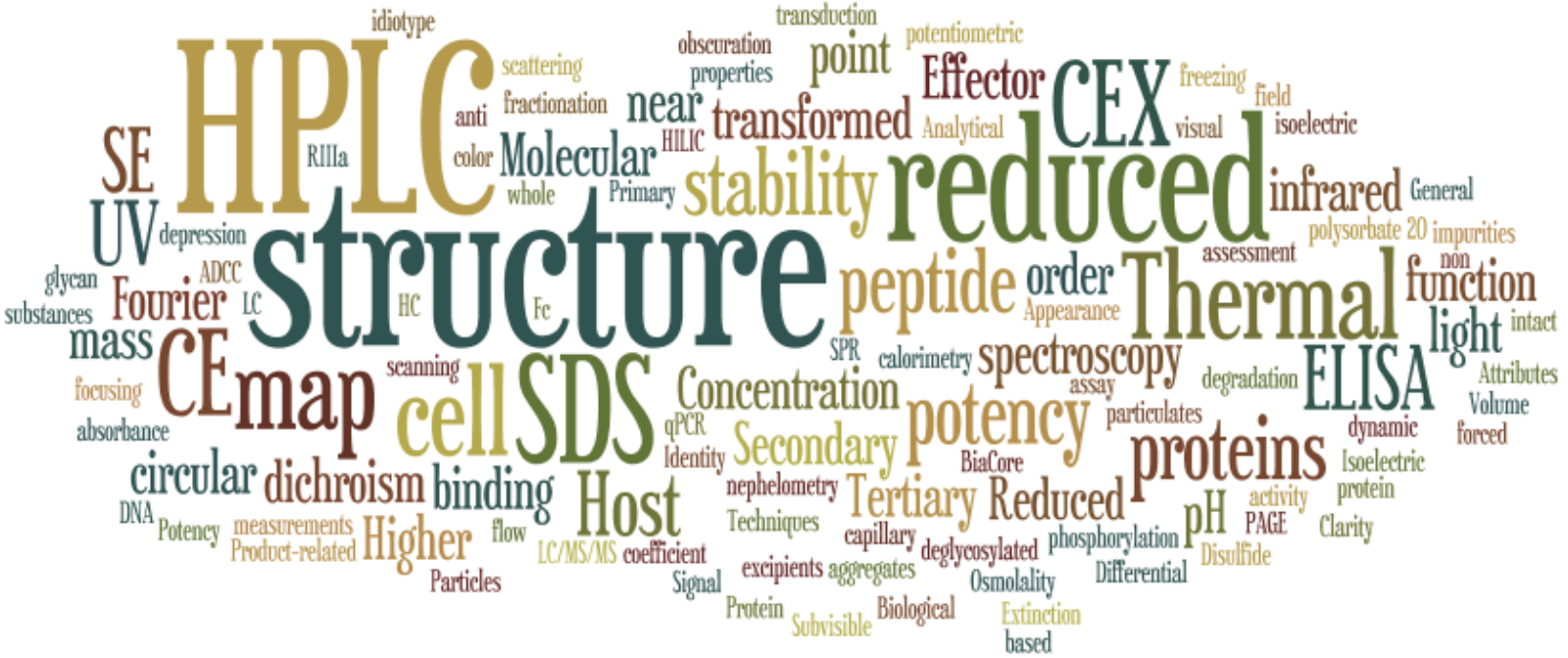
Peptide modifications
- Deamidation
- Succinimide
- Oxidation
- N & C-terminal variants
- Amino acid substitution
- Disulfide isoforms

Folding/Size
- Truncation
- Half molecules
- Dimer
- Multimers
- Aggregates
- Particles

Figure adapted from D. Kelner (Amgen), "Comparability and Biosimilarity: Two Sides of the Same (or a Different) Coin?" presented at IBC Analytical Technologies,San Diego, CA (March 2012)

Typical analytical similarity assessment evaluates 90 to 100 unique attributes

Results from a wide breadth of assay combinations compares the analytical "footprint" of the biosimilar to the reference product.



Is it possible to "match" all attributes?

Figure adapted from J. Liu et al. (Amgen), "Analytical Similarity Assessment of Biosimilars" presented at the Spring ACS Meeting, Dallas, TX (March 2014)

Biosimilar development can use a Quality-by-Design (QbD) approach

QbD for biosimilars

- Assess criticality based on literature & experience
- Characterize reference product quality attributes
- Design biosimilar to minimize differences for high criticality attributes
- Assess potential clinical relevance of remaining differences

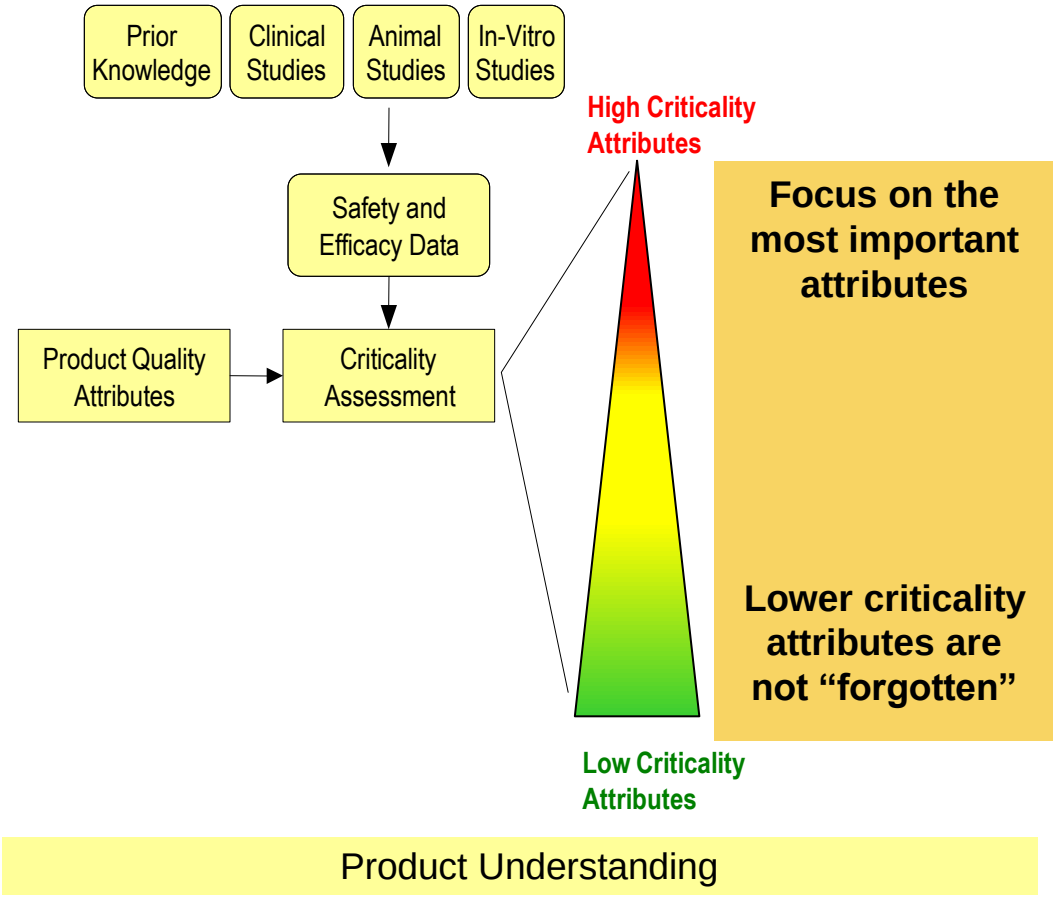


Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

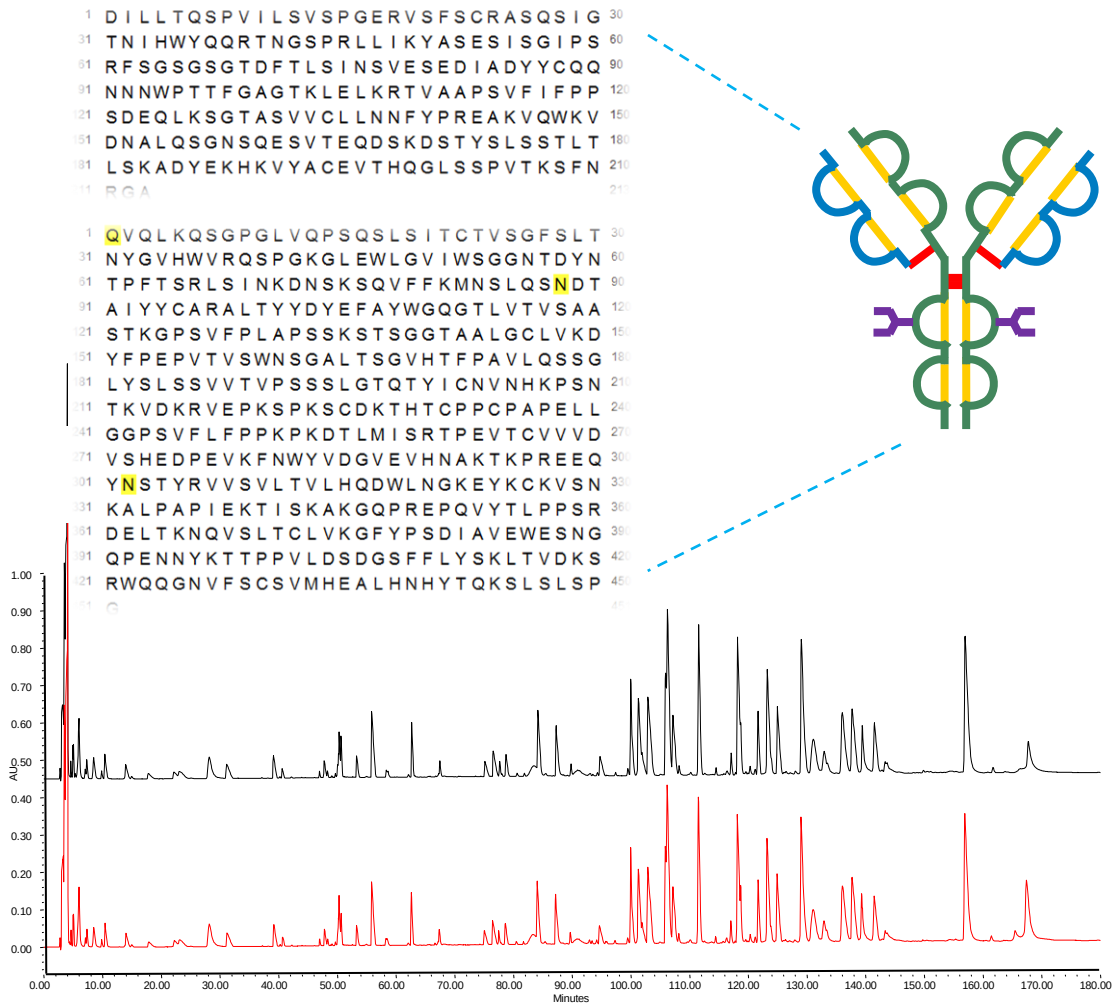
Discussion Topics

- Overview of biosimilar development
- Elements and limitations of analytical studies
- Role of structure-function studies

Analytical studies should assess several aspects of structure

- Primary structure (sequence and linkages)
- Higher order structures (folding, aggregates)
- Covalent modifications (glycosylation and chemical modifications)
- Impurities (product and process)
- Stability profile

Biosimilar product should have identical amino acid sequence to the innovator



Peptide mapping

- 100% sequence confirmation
- Search for any low level amino acid substitution (sequence variant) due to translational errors, misincorporation, or mutation
- Post-translational modifications, such as glycosylation, acetylation, sulfation, phosphorylation, glycation, etc

Amgen unpublished data

Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Primary structure and covalent modifications can be assessed to high fidelity

Mass spectroscopy combined with separation based methods can address many uncertainties

- **Amino acid sequences confirmed to ~100% coverage**
- **Covalent modifications, sequence variants and glycan structures detected to <1% resolution**

Example: LC ESI MS/MS

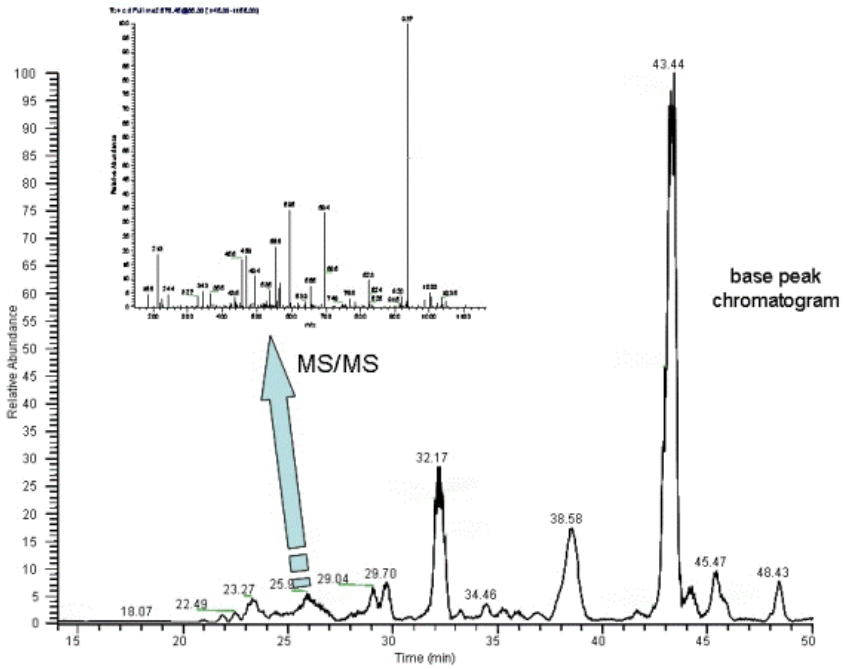


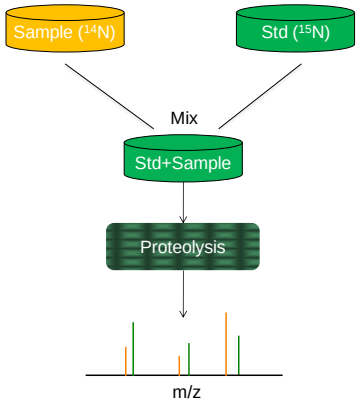
Figure adapted from G. Grampp (Amgen), "Analytical Similarity Assessments" presented at the DIA/FDA Biosimilars Conference, Washington DC (September 2012)

Image obtained from University of Kentucky Mass Spectrometry Facility http://www.research.uky.edu/ukmsf/example2a.html

Advanced mass spectroscopy methods still leave some uncertainties

Examples of some remaining challenges

- Accurate quantitation of minor species
- Identifying and quantifying disulfide bonding patterns
- Accounting for combinatorial effects



Stable Isotope Labeled Internal Standard (SILIS)

Figures courtesy of Jiang et al, PEGS 2011

Correctly folded **Disulfide misfolds 1 and 2**

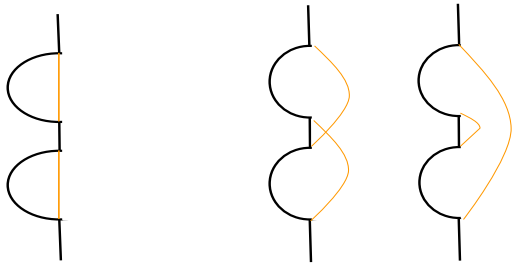
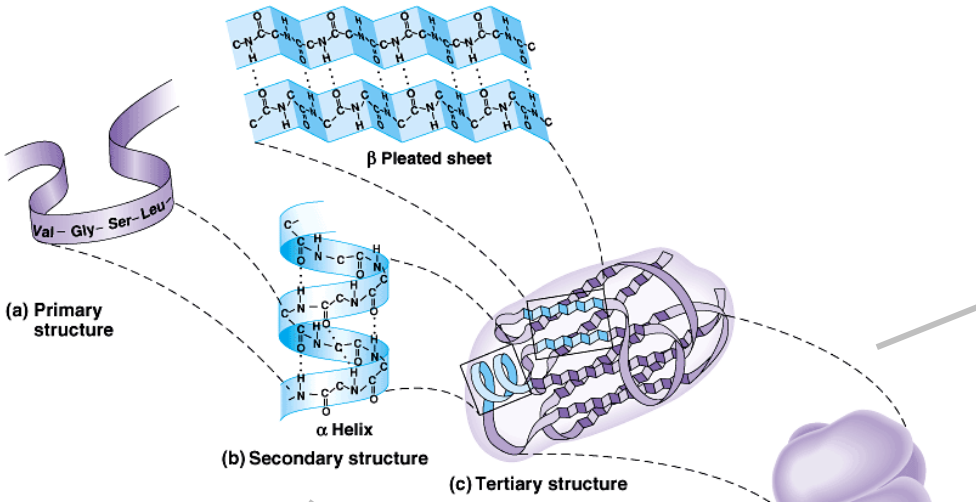
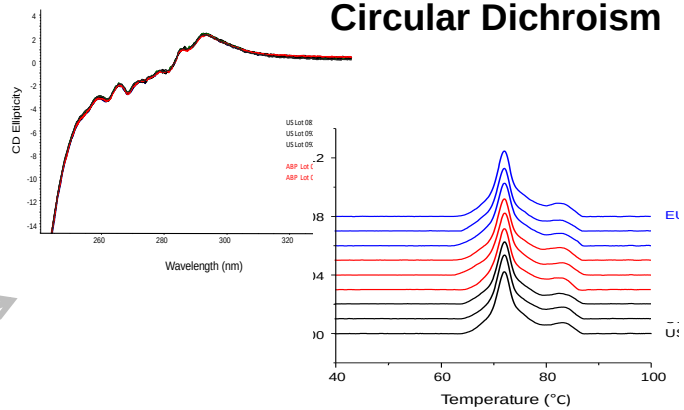
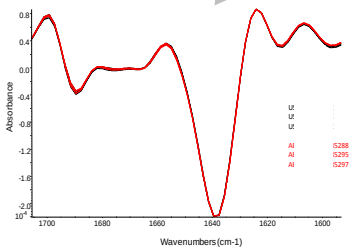


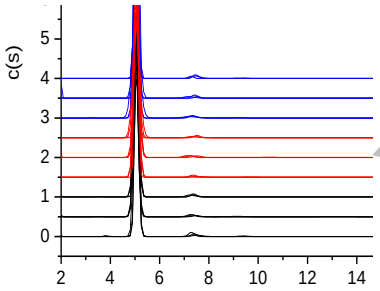
Figure adapted from G. Grampp (Amgen), "Analytical Similarity Assessments" presented at the DIA/FDA Biosimilars Conference, Washington DC (September 2012)

Higher order structure and size variants are characterized by orthogonal methods









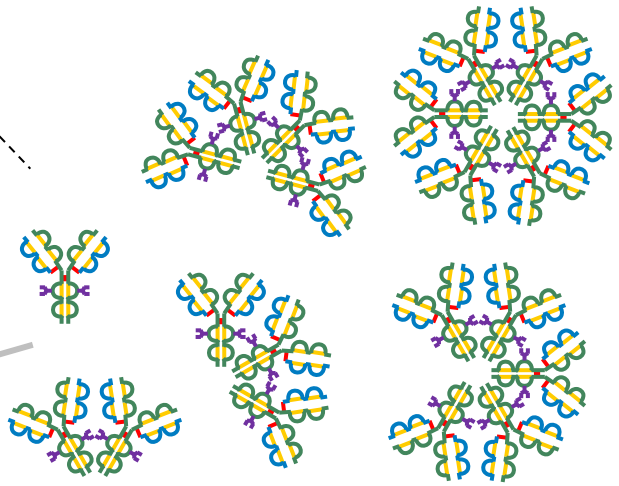


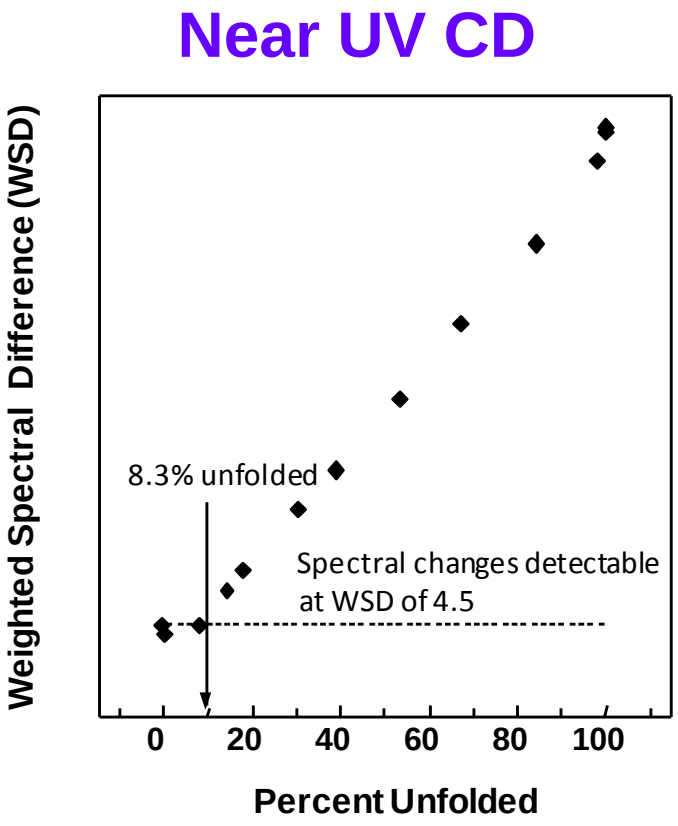
Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Amgen unpublished data

A common limitation of spectroscopic methods is sensitivity to mixtures

Eg, unfolded protein spiked into product

- Limit of detection is 8% by near UV circular dichroism
- How sensitive to partially unfolded species?



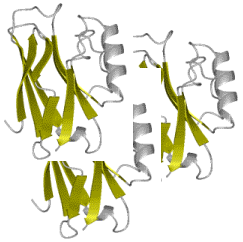
Amgen unpublished data

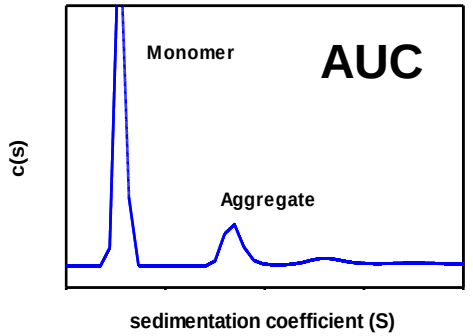
Figure adapted from G. Grampp (Amgen), "Analytical Similarity Assessments" presented at the DIA/FDA Biosimilars Conference, Washington DC (September 2012)

Particulate characterization technology is improving

- Focus on characterizing particles (0.1 μm to 10 μm)
  - Size, composition, quantity, structure
  - Relevance to immunogenicity
- Improving sensitivity, accuracy, and specificity
  - Protein vs. container
  - Emerging nanotechnology-based approaches for < 1 μm particles
- Quantitative and qualitative comparisons remain difficult

Aggregation (<0.1 μm)



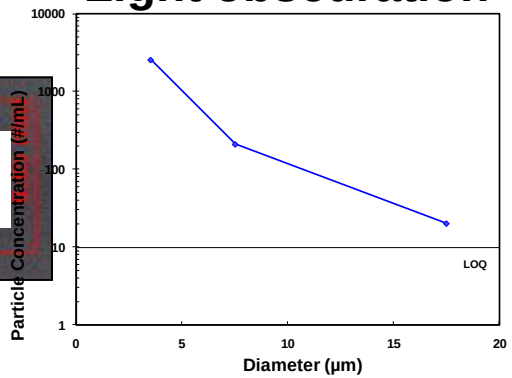


Amgen unpublished data

Particulation (>1 μm)

MFI

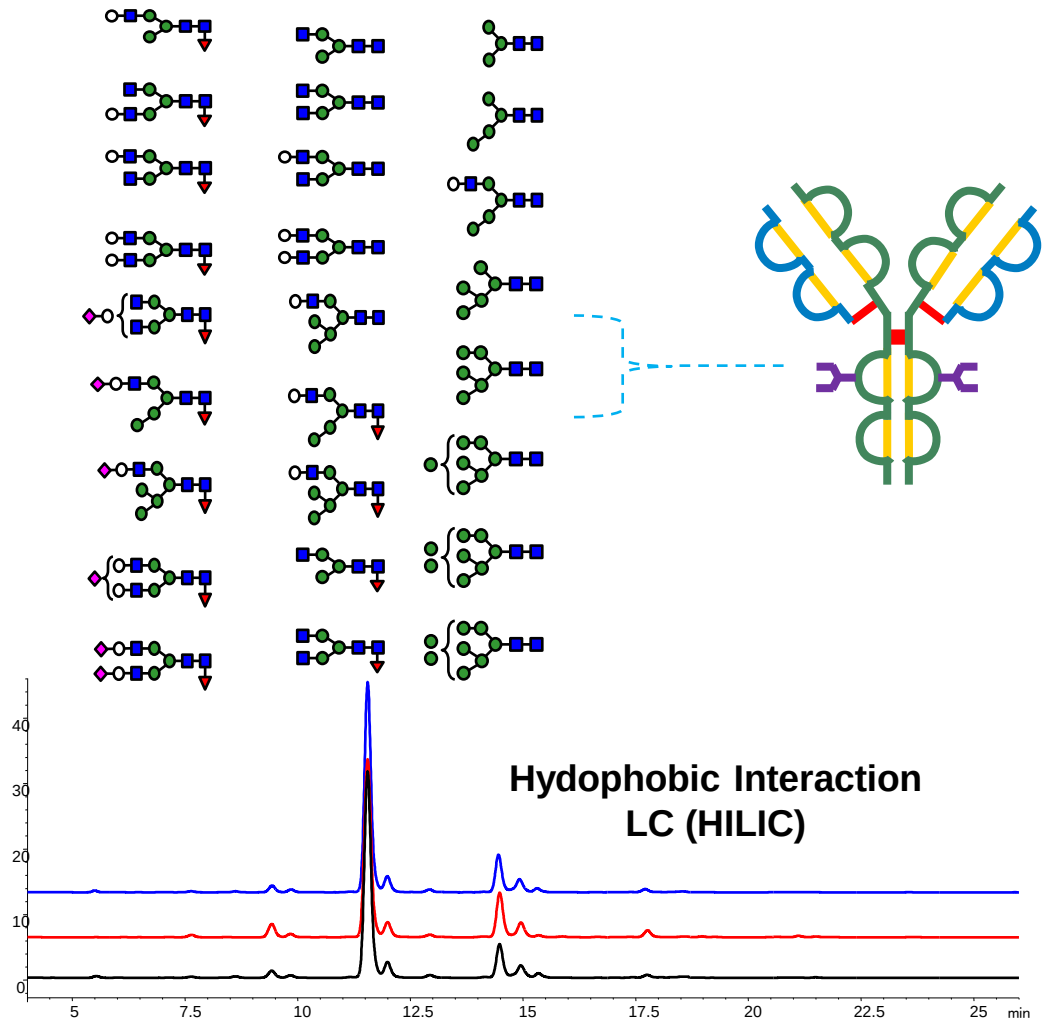
Light obscuration



Amgen unpublished data

Figure adapted from G. Grampp (Amgen), "Analytical Similarity Assessments" presented at the DIA/FDA Biosimilars Conference, Washington DC (September 2012)

Glycosylation is a critical quality attribute that can impact biological functions



Glycan mapping by HILIC and Mass Spectrometry

- Over 25 mAbglycans identified
- Correlate glycan attributes with biological function

Glycan Type	Impact to function
No glycan	No ADCC
Bisecting GN	Increase ADCC
High mannose	Clearance and effector function
TerminalGal	Increase CDC
NANA	Anti-inflammatory
Afucosylated	Increase ADCC

Amgen unpublished data

Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Product isoforms need to be fully characterized using separation methods

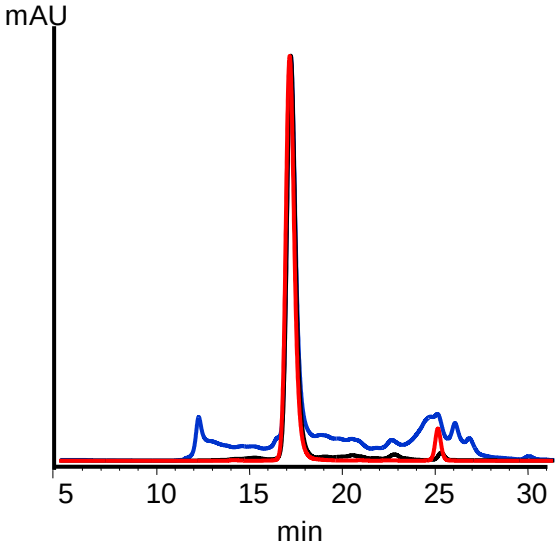
Size variants

- Truncation
- Dimer
- Multimers

Charge and hydrophobic variants

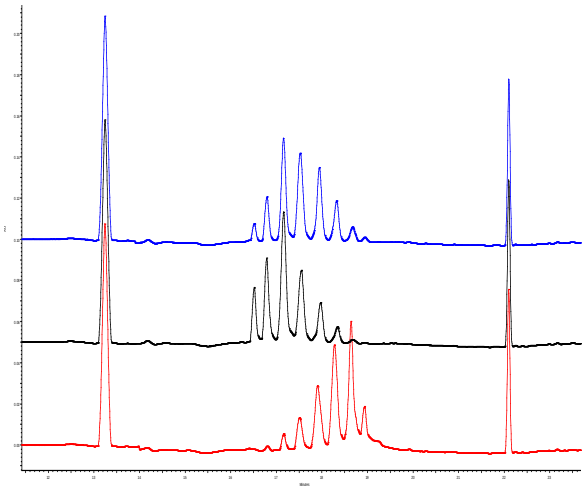
- N-terminal modification
- C-terminal modification
- Deamidation
- Oxidation

Size Exclusion HPLC



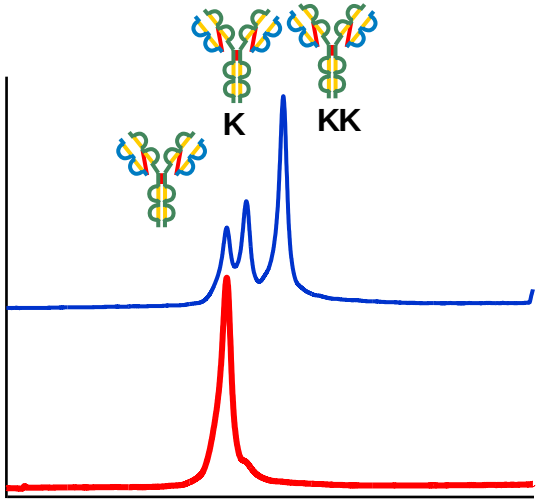
Amgen unpublished data

Isoelectric Focusing



Amgen unpublished data

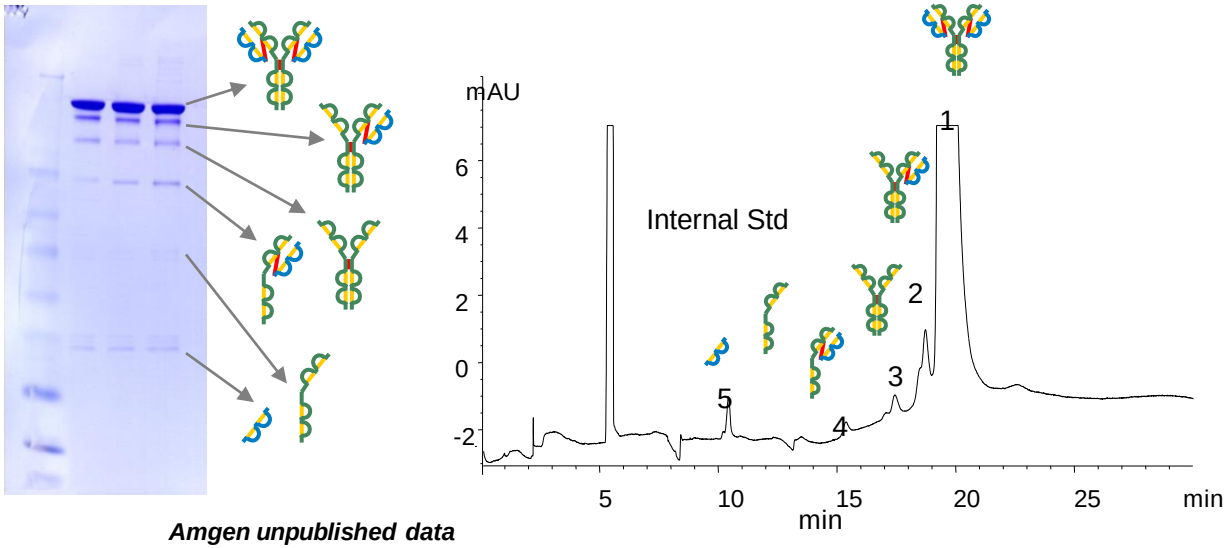
Ion Exchange HPLC



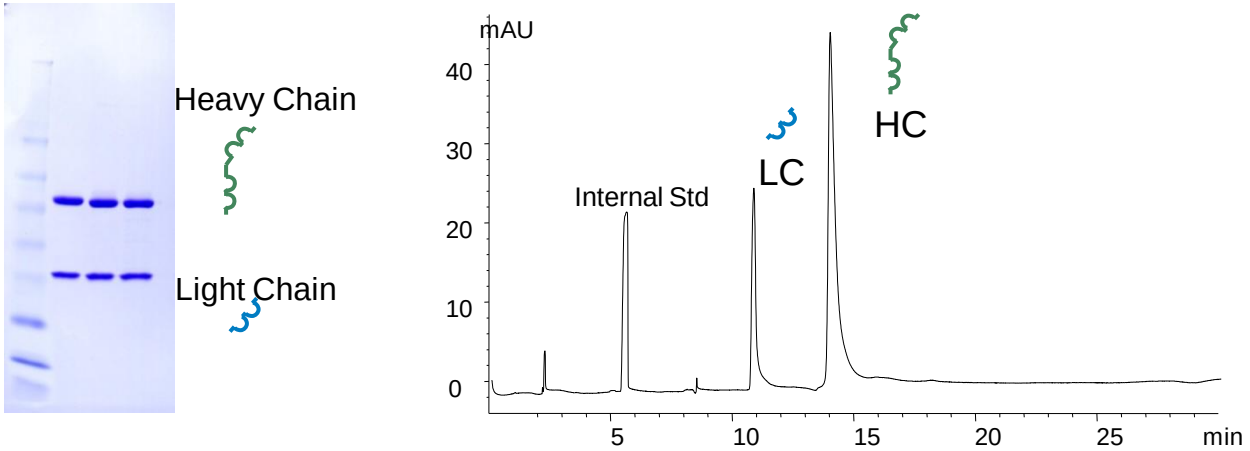
Amgen unpublished data

Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Separation methods also used to examine the integrity of covalent structure



Amgen unpublished data



Non-reducing SDS

- Partial molecules
- Half molecules
- Fragments
- Non-disulfide linked aggregates

Reducing SDS

- Truncation
- Clipped species

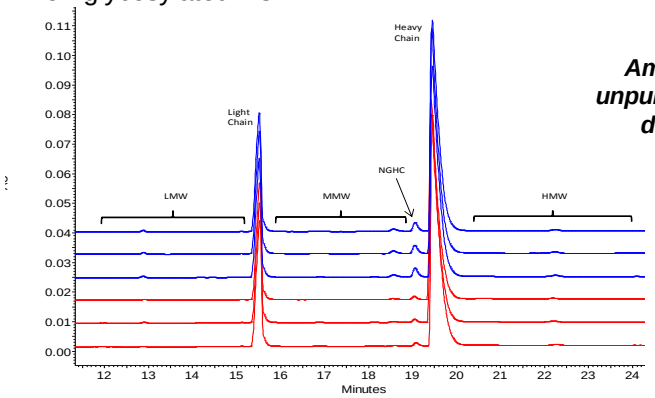
Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Product-related and process-related impurities must be well characterized

- High resolution and orthogonal methods are required to characterize product-related species.

Size variants:

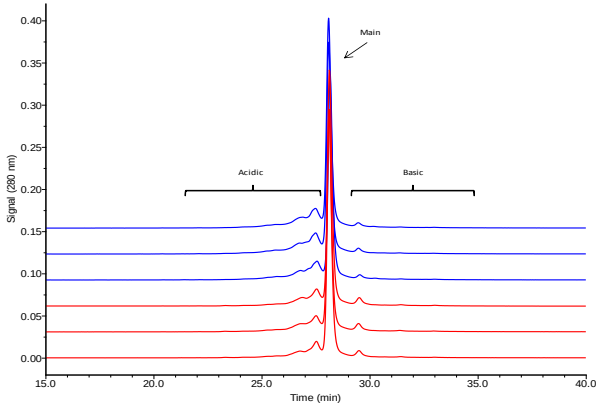
- Truncation
- Dimer
- Multimers
- Clipped species
- Non-glycosylated HC
- Partial molecules
- Half molecules
- Fragments
- Non-disulfide linked aggregates



Amgen unpublished data

Charge and Hydrophobic Variants:

- N-terminal modification
- C-terminal modification
- Deamidation
- Oxidation

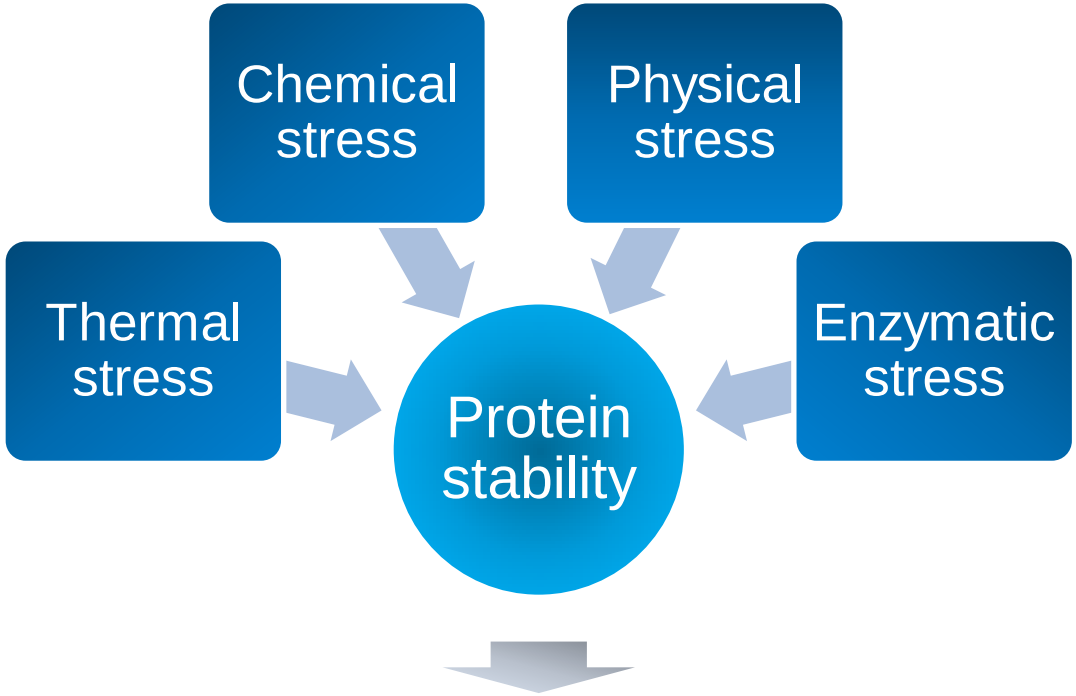


- Process-related impurities (HCP, DNA, leachables, etc) need to be characterized to ensure product quality.
- Particles and aggregates of various sizes need to be evaluated and characterized.

Figure adapted from J. Liu et al. (Amgen), "Analytical Similarity Assessment of Biosimilars" presented at the Spring ACS Meeting, Dallas, TX (March 2014)

Proteins undergo complex degradation and are sensitive to storage and handling

Biosimilar stability is impacted by its manufacturing process and formulation



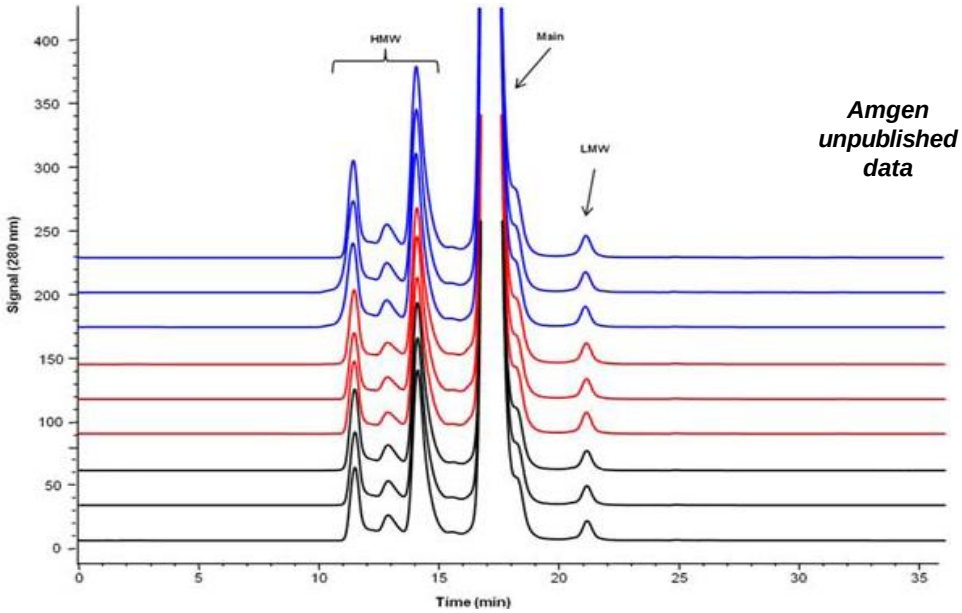
Degradation contributes to eventual loss of biological activity and/or potential immunogenicity

Figure adapted from J. Liu (Amgen), "Analytical Testing and Characterization of Biosimilars" presented at the Health Canada SEB/Biosimilar Scientific Forum, Ottawa, Canada (November 2013)

Forced degradation studies should demonstrate similar stability profiles

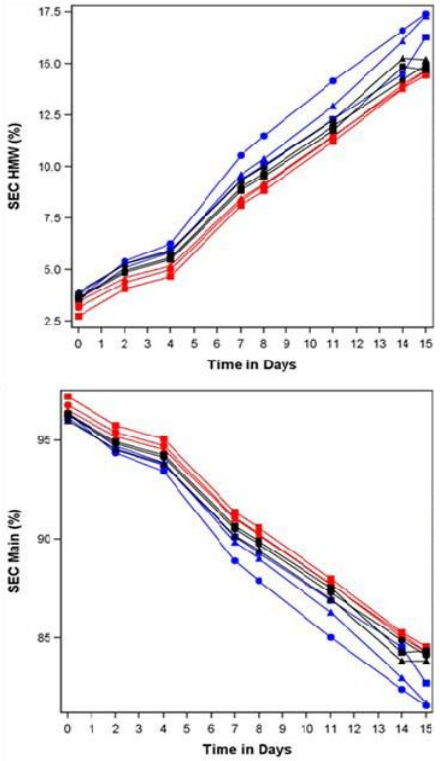
Multiple accelerated thermal stress conditions (25, 40, 50°C) provide a quantitative, reproducible, and sensitive comparison of degradation profiles and rates

- Example: Size Exclusion Chromatography profiles – 50°C, T=15 days



Amgen unpublished data

- Rate comparisons: 0-15 days



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Discussion Topics

- Overview of biosimilar development
- Elements and limitations of analytical studies
- **Role of structure-function studies**

Structural comparisons leave residual uncertainties

Sources of uncertainty	Potential consequences
Assay limitations (limit of detection, specificity, etc.)	Unobserved differences could potentially impact efficacy or safety
Lot to lot variability and population statistics	Equivalence of means does not prove that individual lots are biologically equivalent
Observed differences in critical attributes	Could impact safety or efficacy if differences are large enough
Observed differences in less criticalattributes	• Are assumptions about criticality correct? • Could combinations of attributes become significant?

Functional studies are the first step in addressing these residual uncertainties

Why functional characterization?
Part 1: Required by regulators

- Functional characterization required
  - To confirm quality and potency of the product
  - To address limitations of structural assays
  - To confirm similar mechanism(s) of action
    - presence of expected function, absence of new function
    - specificity of target binding

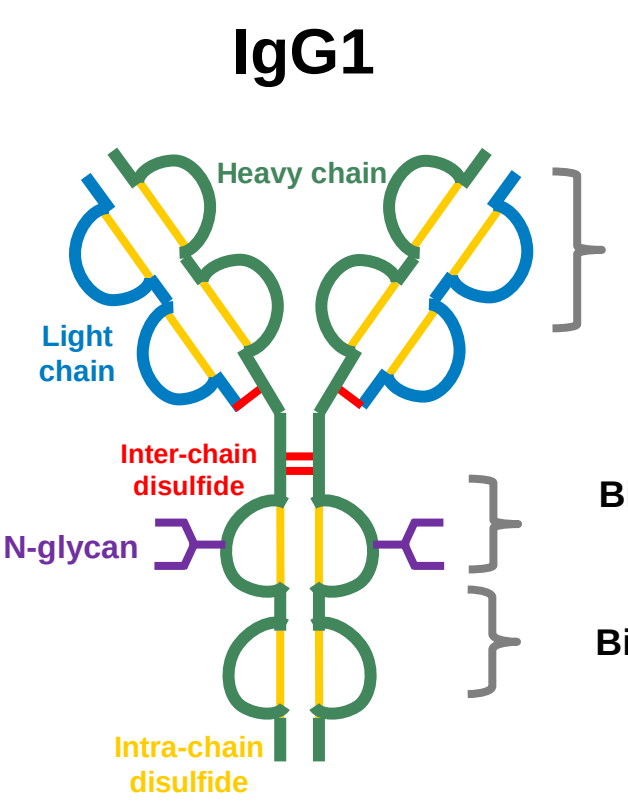
- *Relevant passage from FDA guidance*

*"Depending on the structural complexity of the protein and available analytical technology, the **physicochemical analysis may be unable to confirm the integrity of the higher order structures**. Instead, the integrity of such structures can be inferred from the product's biological activity." (Emphasis added)*

FDA Draft Guidance, Quality Considerations in Demonstrating Biosimilarity to a Reference Product, February 2012

Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

Matching all biological and functional properties is essential

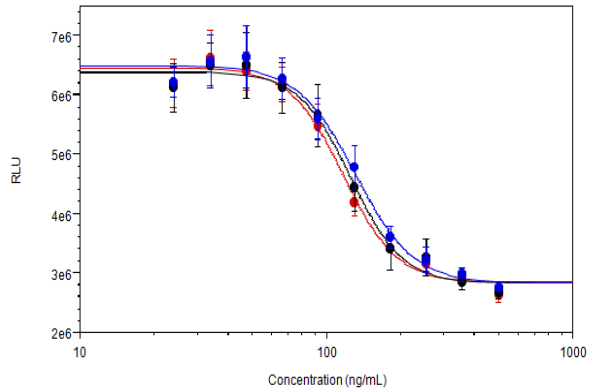


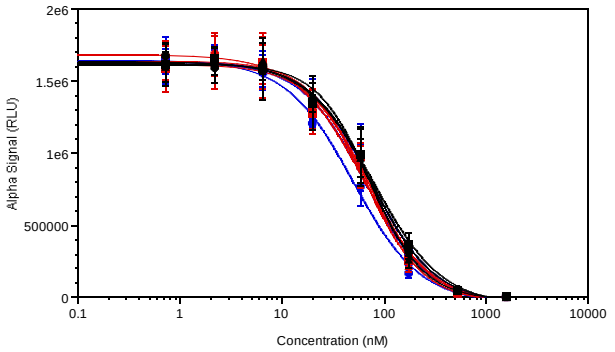
Target binding to Complementarity-Determining Regions (CDRs)

Binding Region for FcγR (ADCC, CDC)

Binding Region for FcRn (PK ½ life)

Amgen unpublished data





Biological functions are dependent on the target antigen and the class of antibody

Figure adapted from J. Liu et al. (Amgen), "Analytical Similarity Assessment of Biosimilars" presented at the Spring ACS Meeting, Dallas, TX (March 2014)

Why functional characterization?
Part 2: May be essential to justify differences

QbD for biosimilars

- Assess criticality based on literature & experience (where available)
- Minimize differences for high criticality attributes

- Perform structure-function studies to assess remaining differences
- Relate findings to potential clinical impact

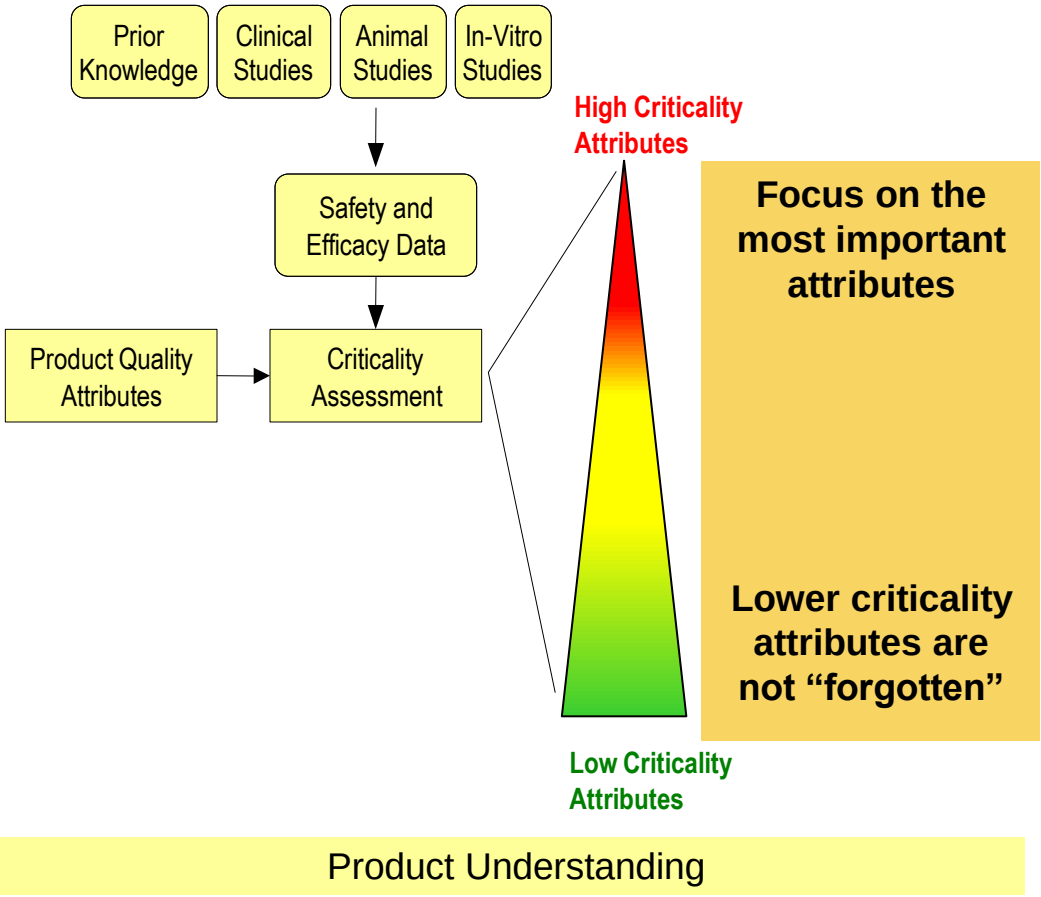
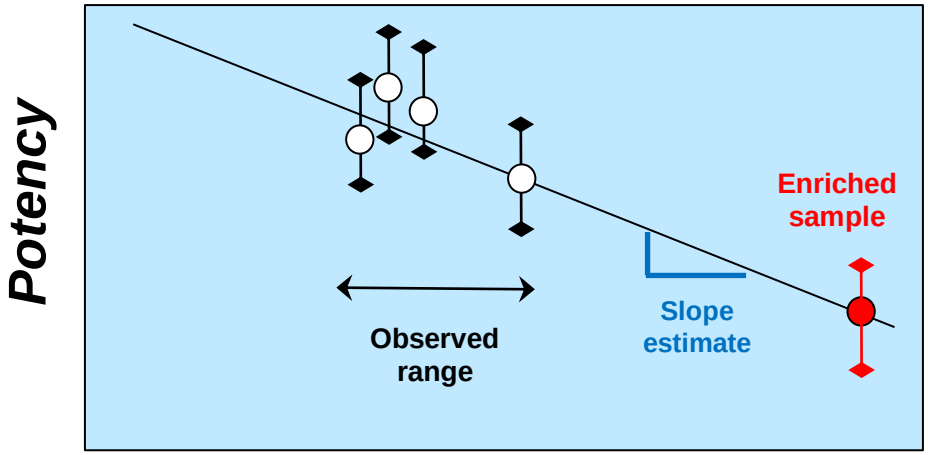


Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

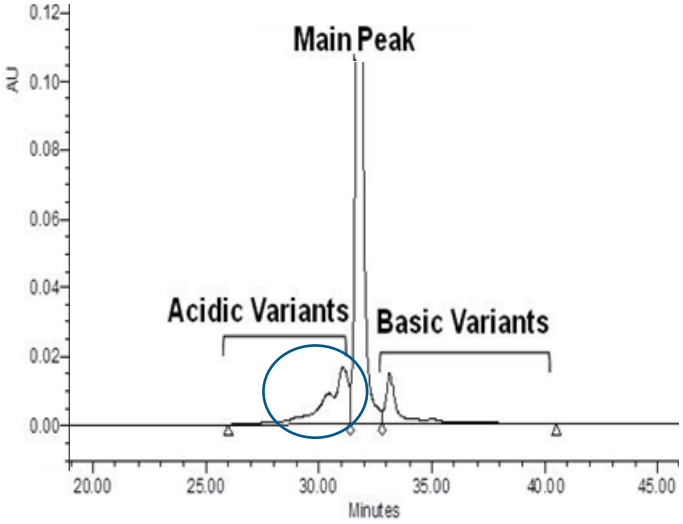
Prepared samples can increase sensitivity of structure-function studies



Notional data for illustration purposes only

Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

Cation exchange profile of a mAb



Charge Variants	% Relative Potency
Acidic variants	82
Main peak	101
Basic variants	84

Amgen unpublished data

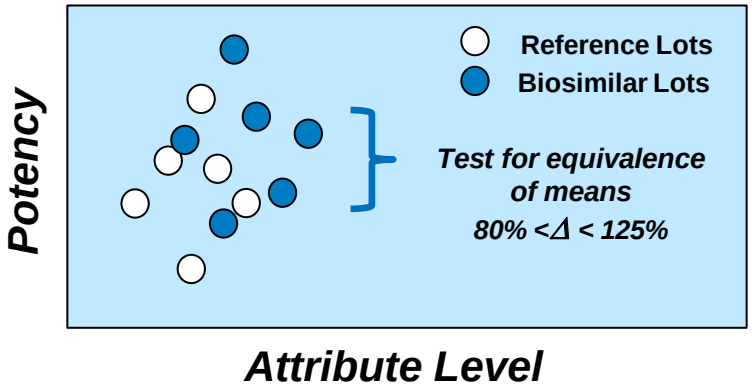
Improved estimate of slope informs potential criticality and permitted magnitude of differences

Studies must provide relevant conclusions

1) Evaluate in vitro functional data

a) Test functional equivalence of actual batches

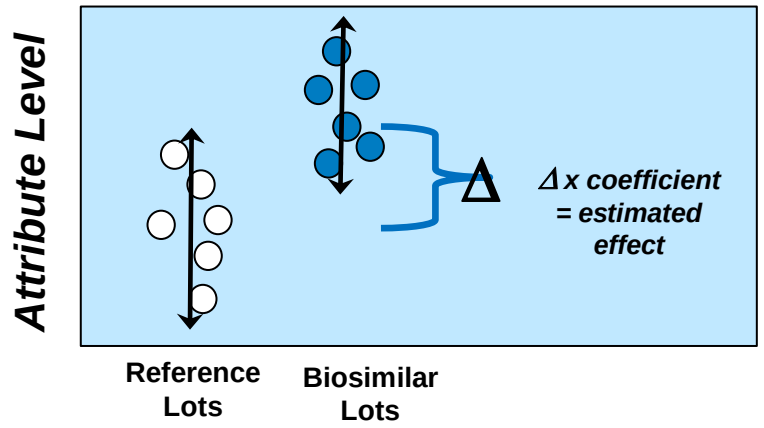
Is a 25% difference *really* acceptable?



Notional data for illustration purposes only

b) Relate attribute difference to parameters from structure–function studies

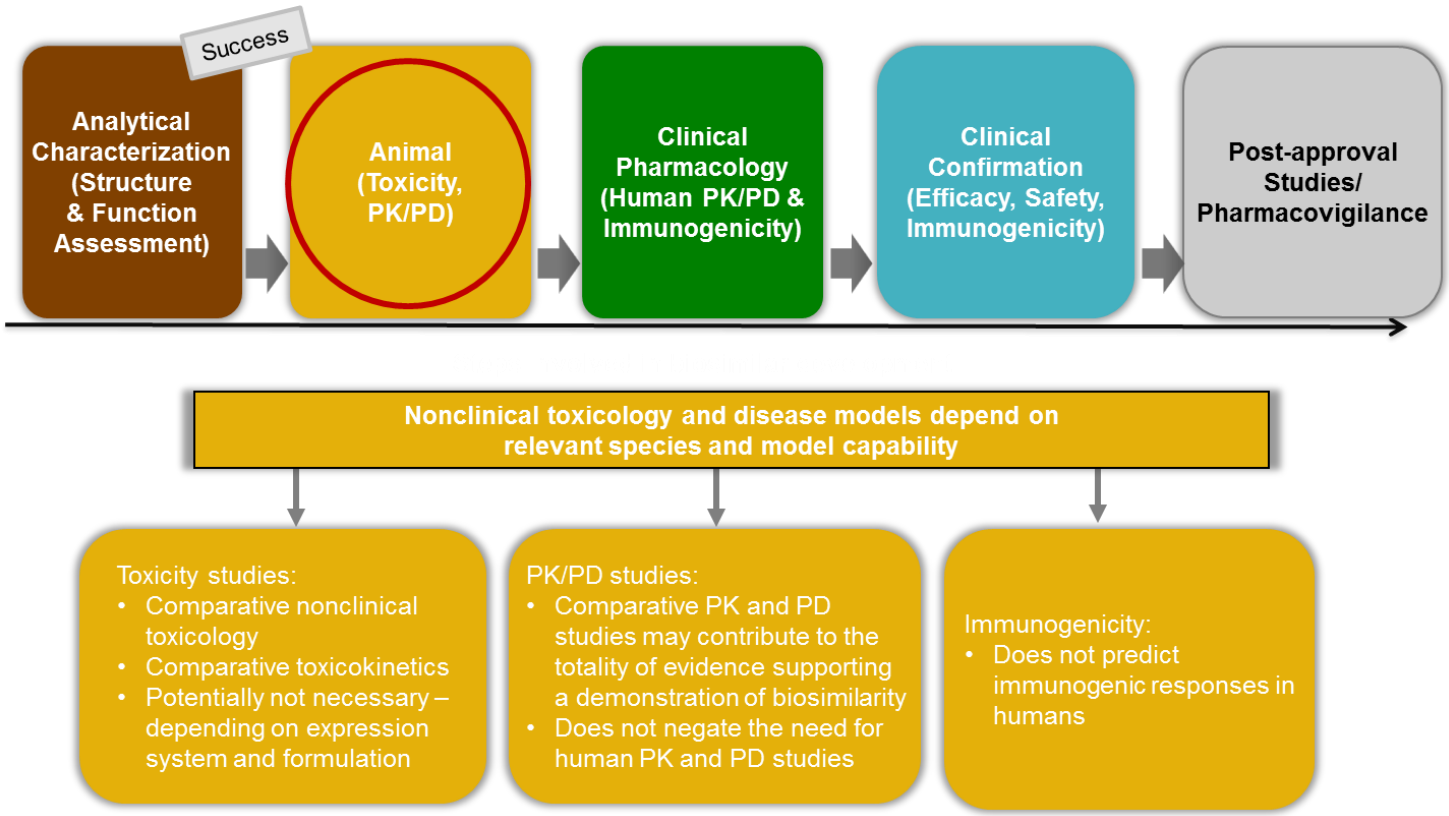
- Measured difference in means
- Estimated quantitative effect
- Relate to clinically meaningful differences



Notional data for illustration purposes only

Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

Nonclinical Studies



US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015.

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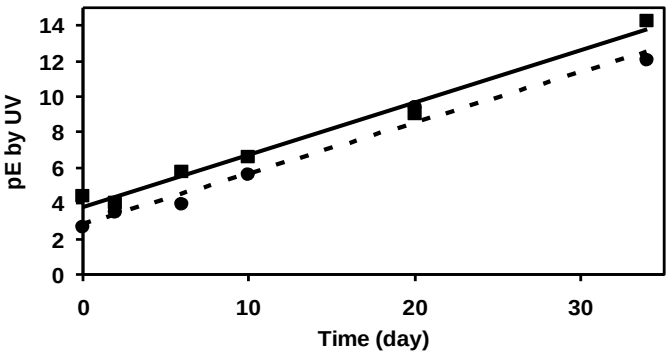
Studies must provide relevant conclusions

2) Evaluate PK and drug metabolism where feasible

- Serum incubation in vitro: is a variant formed under physiologic conditions?

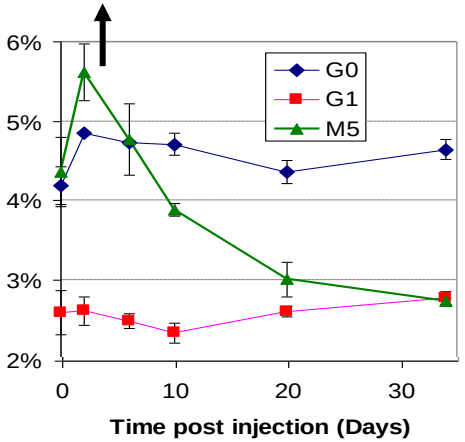
In vivo vs. in vitro conversion of Glu to pyroGlu



Adapted from Liu et al. 2011, J.Biol. Chem. 286, 11211-11217

- Product recovery from PK samples

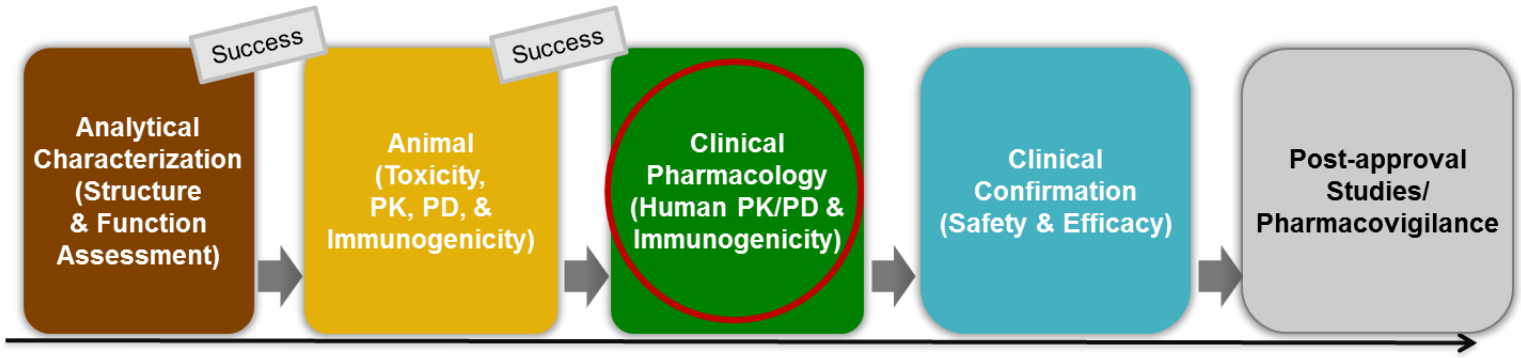
M5 increases initially



followed by decrease

Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

Pharmacology: pharmacokinetics and pharmacodynamics



Pharmacology is critical component of biosimilar evaluation[1]

- PK (pharmacokinetics) in healthy volunteers is generally the most sensitive population to assess kinetic similarity[2]
- Equivalent PK (generally 90% CI to be within 80–125% as standard) establishes same dose as reference product assuming equivalent potency and biologic activities[2]
- PD with dose–response equivalence can be used to infer clinical efficacy if sensitive and relevant marker is available[1]

1. US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015. 2. US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm397017.pdf. Accessed September 2015.

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Small effects can combine in unexpected ways

Process change case study

- **Change resulted in shifts in 2 attributes (see figure)**
- **Bioassays predicted equivalent potency**
- **Equivalent PK shown in human clinical study**
- **Potency difference detected in clinical PD study**
- **Post hoc studies with prepared fractions identified additive effects on potency**

In vitro potency of prepared fractions

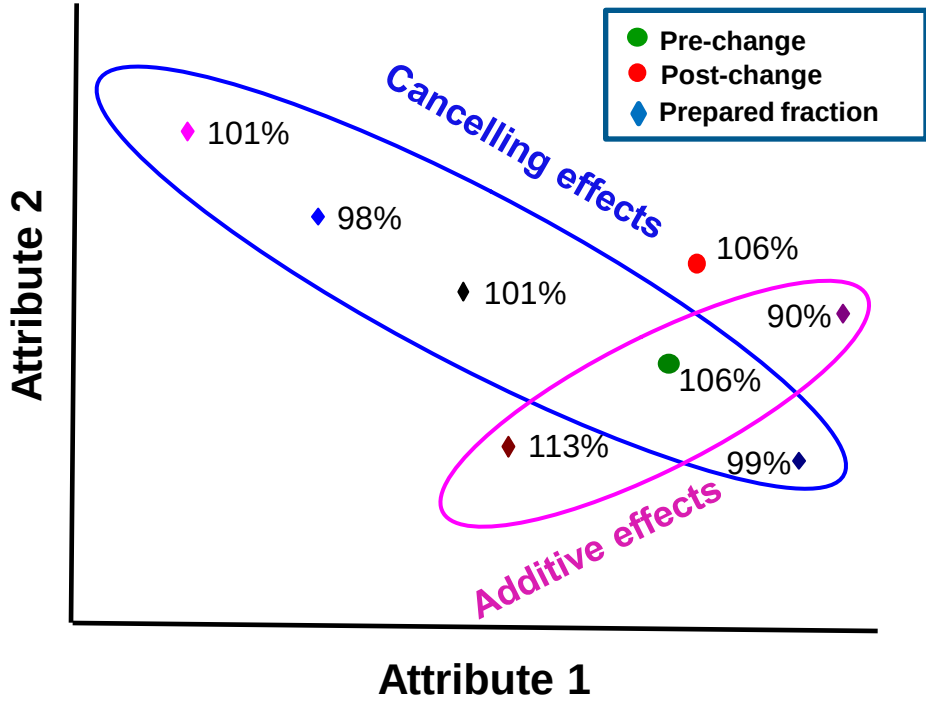


Figure adapted from G. Grampp (Amgen), "Challenges of Structure-Function Studies for Assessing Similarity" presented at the Spring ACS Meeting, New Orleans, LA (April 2013)

Amgen unpublished data

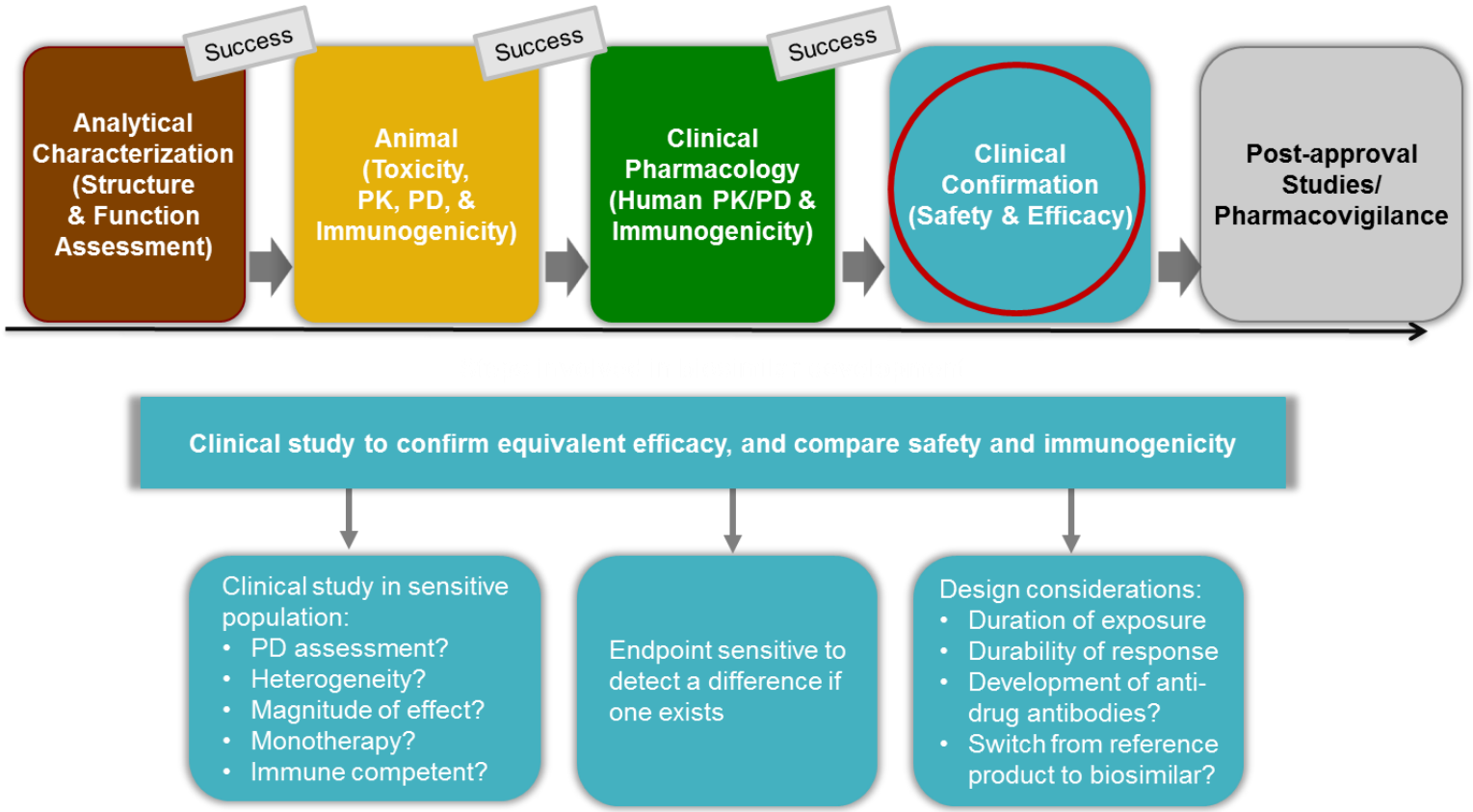
Additional challenges in structure-function studies

- Predicting human PK/PD
  - Animal studies may not account for species specific clearance mechanisms
  - Insufficient power due to small number of animals
- Predicting human immune response
  - In silico, in vitro, and in vivo methods are insufficient to rule-out clinically relevant differences

Summary and Conclusions

- Analytical advances permit high resolution similarity assessments for many attributes
  - Higher order structure and particle assessments still subject to uncertainty
  - Orthogonal approaches partially compensate for lower sensitivity
- Assessing impact of differences remains challenging
  - Not all clinically relevant effects can be evaluated pre-clinically (e.g., PK and immunogenicity)
  - Small effects and combinations difficult to assess

Comparative clinical safety, efficacy, and immunogenicity



US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015.

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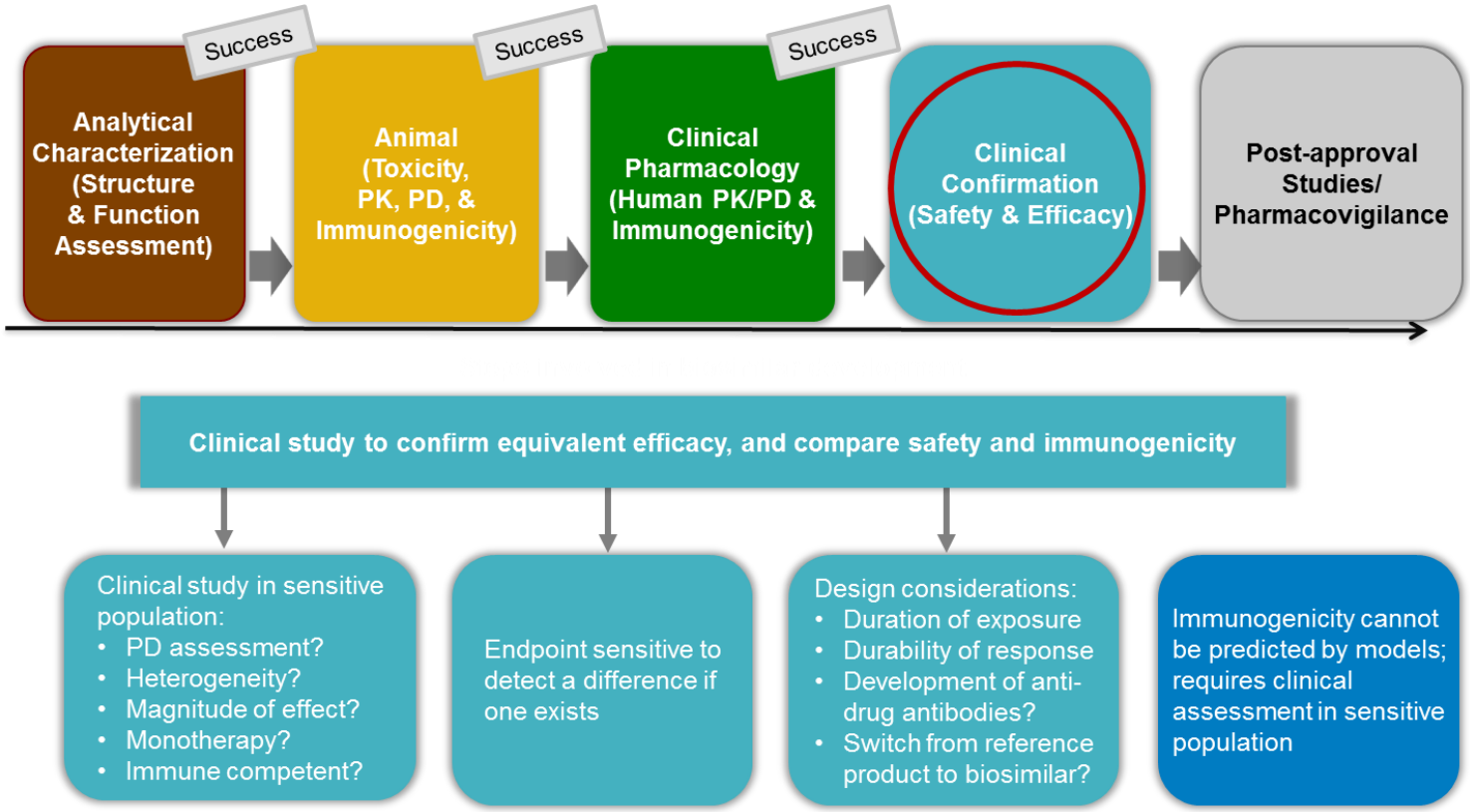
Comparative clinical safety, efficacy, and immunogenicity



US Food and Drug Administration. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm291128.pdf. Accessed September 2015.

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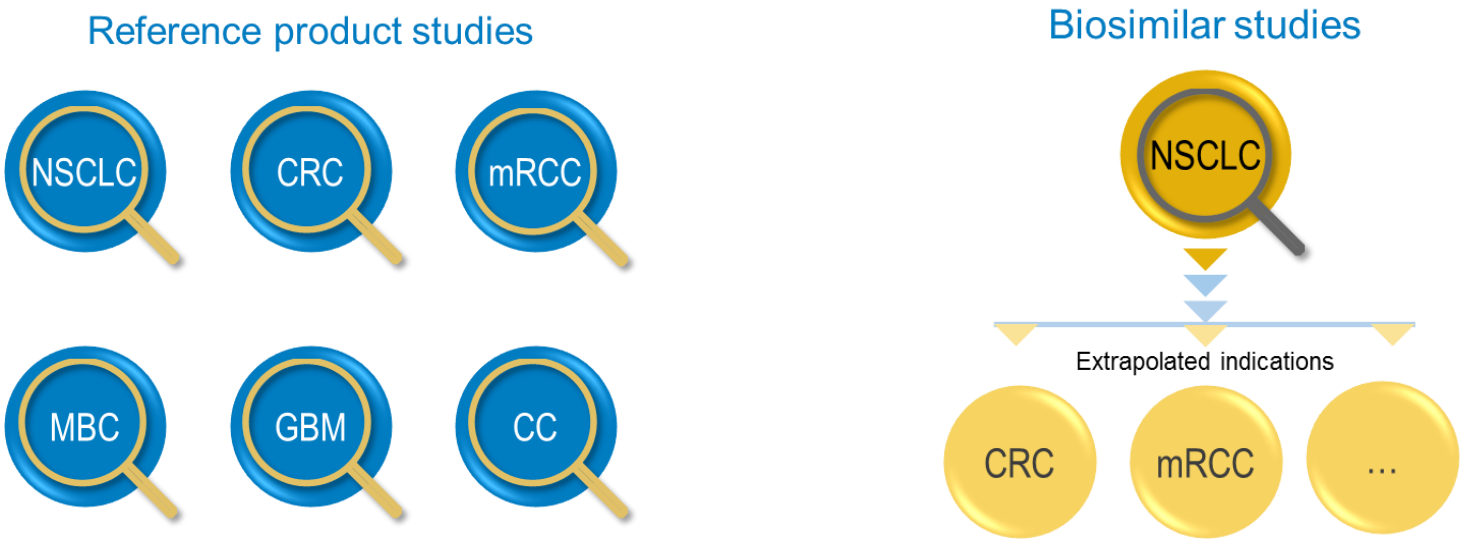
Extrapolation of Indications

A proposed biosimilar product may be licensed in one or more additional indications for which the reference product is licensed, <u>if appropriate scientific justification is provided</u>



CRC = colorectal cancer; mRCC = metastatic renal cell carcinoma; MBC = metastatic breast cancer; GBM = glioblastoma multiforme; CC = cervical cancer.

CBER. *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. Guidance for Industry*. Silver Spring, MD: FDA. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf. Published April 2015. Accessed September 27, 2015.

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Extrapolation of Indications Requires Scientific Justification

Scientific Justification Should Establish

- MOA in each indication*
- PK, PD, and immunogenicity in different patient populations
- Differences in expected toxicities in each indication
- Any other factor that may affect safety or effectiveness in each indication

Health authorities may have differing perspectives on what evidence is sufficient to support extrapolation

*MOA in each indication may include target/receptors for each relevant activity/function; binding, dose/concentration of response, and pattern of molecular signaling upon engagement of target; relationship between product structure and target/receptor interactions; and location and expression of target/receptors.

CBER. *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. Guidance for Industry*. Silver Spring, MD: FDA. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf. Published April 2015. Accessed September 27, 2015.

From a Quality Perspective, Amgen is Able to Match ~100 Critical Attributes Necessary to Show Biosimilarity

Amgen Biosimilar Attributes Compared to U.S. and EU Reference Product

- General Properties
- Primary Structure
- High-Order Structure
- Biological
- Product-Related Substances and Impurities
- Process−Related Impurities
- Particles and Aggregates
- Thermal-Forced Degradation

Product Example

	ABP vs. U.S. Reference Product	ABP vs. EU Reference Product
Attributes Matched	91	93
Attributes Not Matched but Not Critical	4	2
Attributes Not Matched and Critical	0	0

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Thank you!

Questions?