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Uniquely Flexible CMO™

Developing Biosimilars and Biobetters

Parenteral Drug Association Metro Chapter

Somerset, NJ *January 29, 2014*

Michiel E. Ultee, Ph.D, CSO

Gallus Biopharmaceuticals, Princeton, NJ

Gallus Biopharmaceuticals, LLC

- Well-established CMO with decades of experience
  - Mab's
  - Fusion proteins
  - Other recombinant proteins
  - All mammalian-cell culture
- Full service
  - Vial of Cells → Vials of Drug Product
  - Process Development & Manufacturing
  - Production, Purification, Filling
- Pure CMO -- Have none of our own products
- Located in Princeton, NJ and St. Louis, MO

Attaching buffers to chromatography skid

What are Biosimilars & Biobetters?

- **Biosimilar**
- Recombinant protein therapeutics that resemble but are not identical to the original or reference product, *i.e.,* a generic biologic drug.
- Closely resembles reference product in safety, purity and potency
- Shows no clinically meaningful differences

- **Biobetter**
- Enhanced version of the reference product. Clinically meaningful differences are expected.
- Potentially an improved product in terms of efficacy, dosing, potency, etc. --- *i.e*., a 2nd generation biologic drug

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Why Biologics ≠ Standard Generic Drugs

- They are polymers of much larger size (100-5000X) and complexity than chemical drugs
- They are produced by living cells -- It is almost impossible for the identical protein to be made by two different cell lines in different locations.
- All therapeutic proteins are a mix of closely related variants -- Even run-to-run differences exist at a single manufacturer.

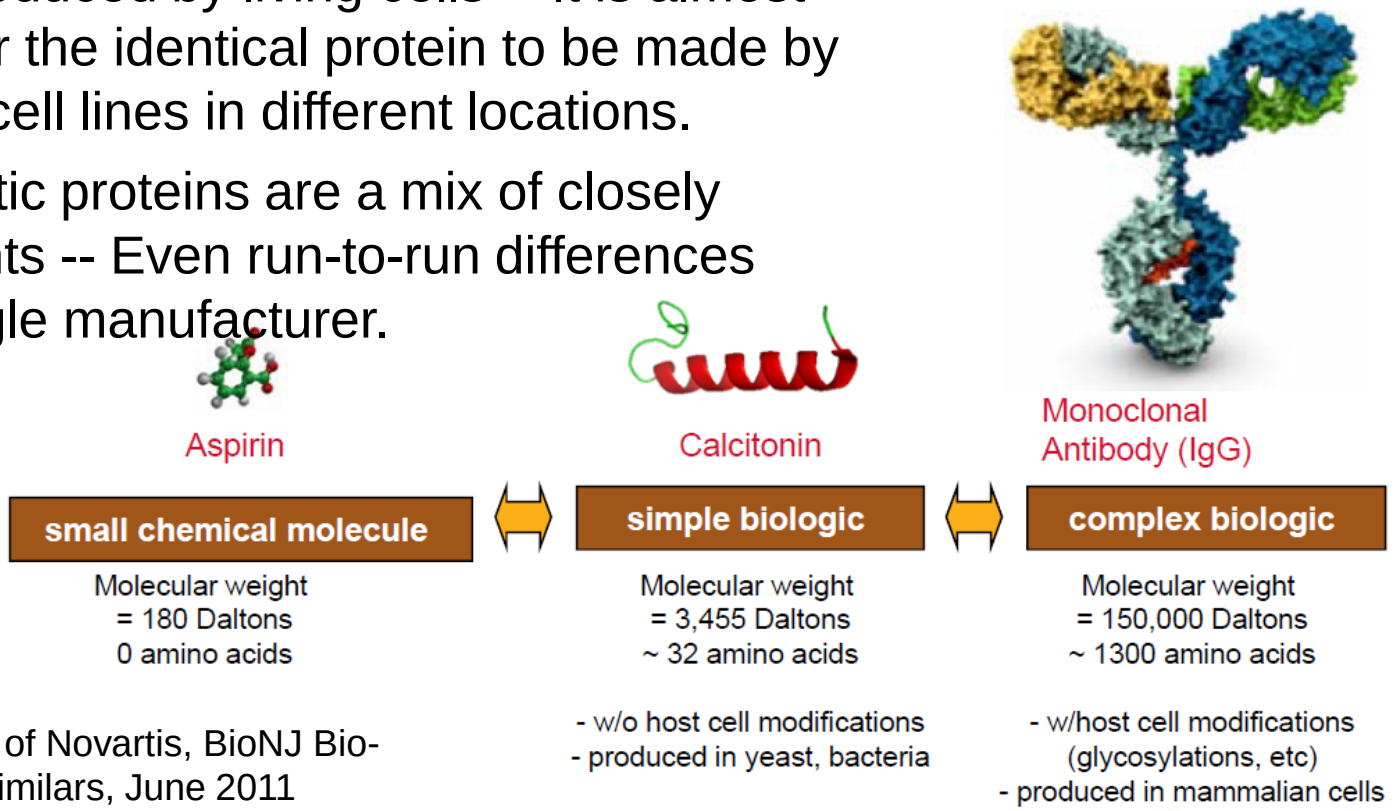

Figure from Shefali Kakar of Novartis, BioNJ Bio-breakfast Briefing on Biosimilars, June 2011

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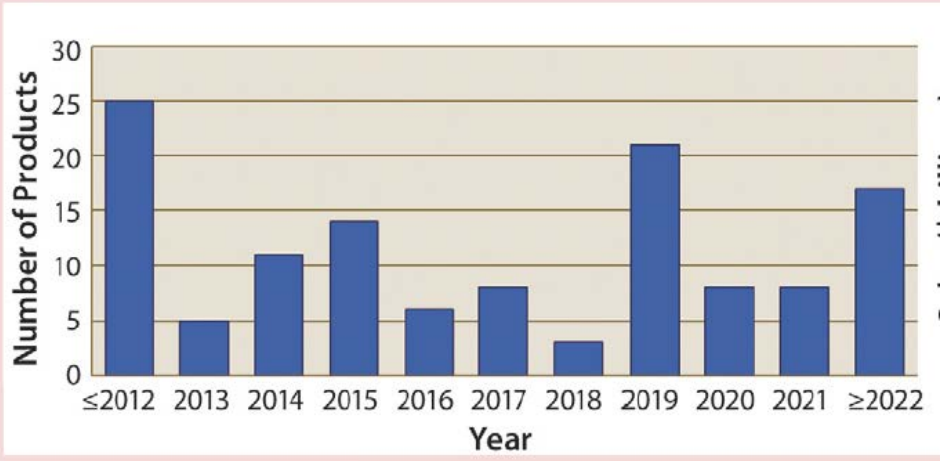
Why the Interest in Biosimilars?

- **Follow the Money!** Over $70B in biologics revenues open to competition from biosimilars in the next 5 years as patents expire

Figure 1: Biosimilar launchable dates



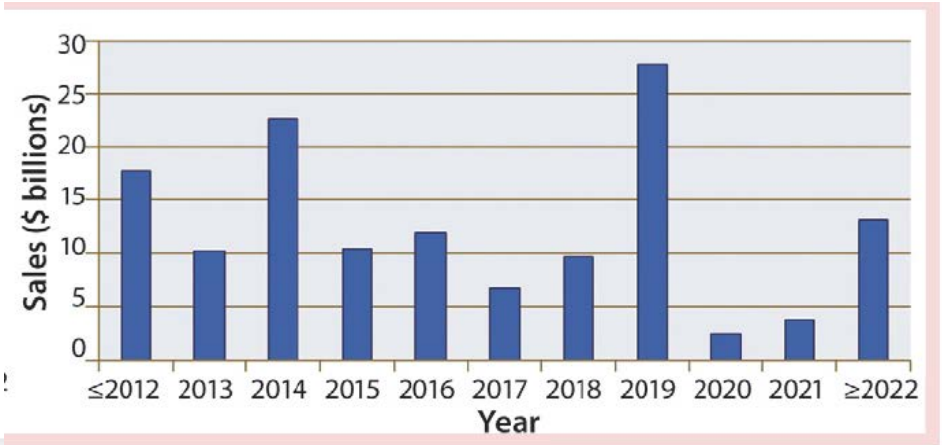


Table 2: Some major recombinant products and classes with biosimilars and biobetters currently in development

Product or Product Class	Sales (US$billions)	Biosimilars	Biobetters
Humira (adalimumab)	$9.27	13	7
Remicade (infliximab)	$8.90	9	9
Enbrel (etanercept)	$7.87	21	8
Rituxan (rituximab)	$7.29	30	17
Herceptin (transuzumab)	$6.40	24	12
Lantus (insulin glargine)	$6.40	5	2
Avastin (bevacizumab)	$6.26	14	9
Neulasta (pegfilgrastim)	$4.10	14	9
Lucentis (ranibizumab)	$3.72	2	2
Aranesp (darbepoetin alfa)	$3.00	4	2
Epogen/Procrit (epoetin alfa)	$3.73	69	26
Novoseven (coagulation factor VIIa)	$1.50	8	12
Neupogen (filgrastim)	$1.44	52	17
Insulin and analogs		40	53
Tumor necrosis factors (MAbs/inhibitors)		44	19
Interferons (alfa)		55	48
Interferons (beta)		23	23
Somatropins		28	17
Factors VIII		4	21
MAbs and antibody fragments		145	91
Cancer-targeted proteins (non-MAb)		264	159
Cancer-targeted MAbs		77	59

From Ronald Rader, *Bioprocess Intl 11(6)s (June 2013)*

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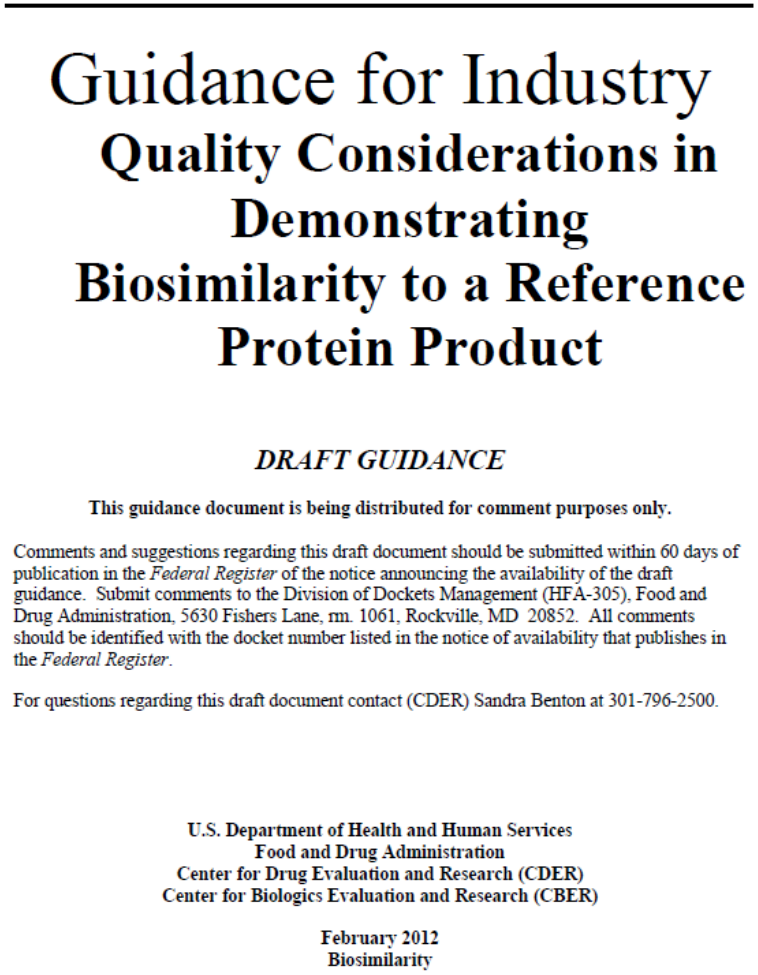
How did Biosimilars Come About?

- Patient Protection and Affordable Care Act of March 23, 2010, *aka* “Obamacare”.
- Directed FDA to create an abbreviated pathway for biological products that are demonstrated to be “biosimilar” to or “interchangeable” with an FDA-licensed biologic.

President Obama signs Patient Protection and Affordable Care Act

Regulatory Progress

- Feb 9th, 2012: FDA Issues Draft Guidance Document on Biosimilars
- Sets for key requirements for biosimilarity
- Primary sequence of protein (amino-acid sequence) must be identical
- "Totality of the Evidence" approach will be used by FDA, with comprehensive analytical analyses, animal and clinical data, to assess biosimilarity
- But, as of today (1/29/2014), no Biosimilars have received FDA approval



Guidance for Industry

Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Sandra Benton at 301-796-2500.

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)

February 2012
Biosimilarity

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Progress In Europe

- Well ahead of US. Initial Guidelines issued in 2005, 7 years before FDA
- Already 14 Biosimilars approved and on market, mostly smaller and simpler proteins.
- First more complex proteins, Monoclonal Antibodies (MAB), approved in Sept 10, 2013, with Biosimilar for Remicade (Infliximab) to Hospira & Celltrion.

PMLiVE

First biosimilar mAb gets the nod in EU

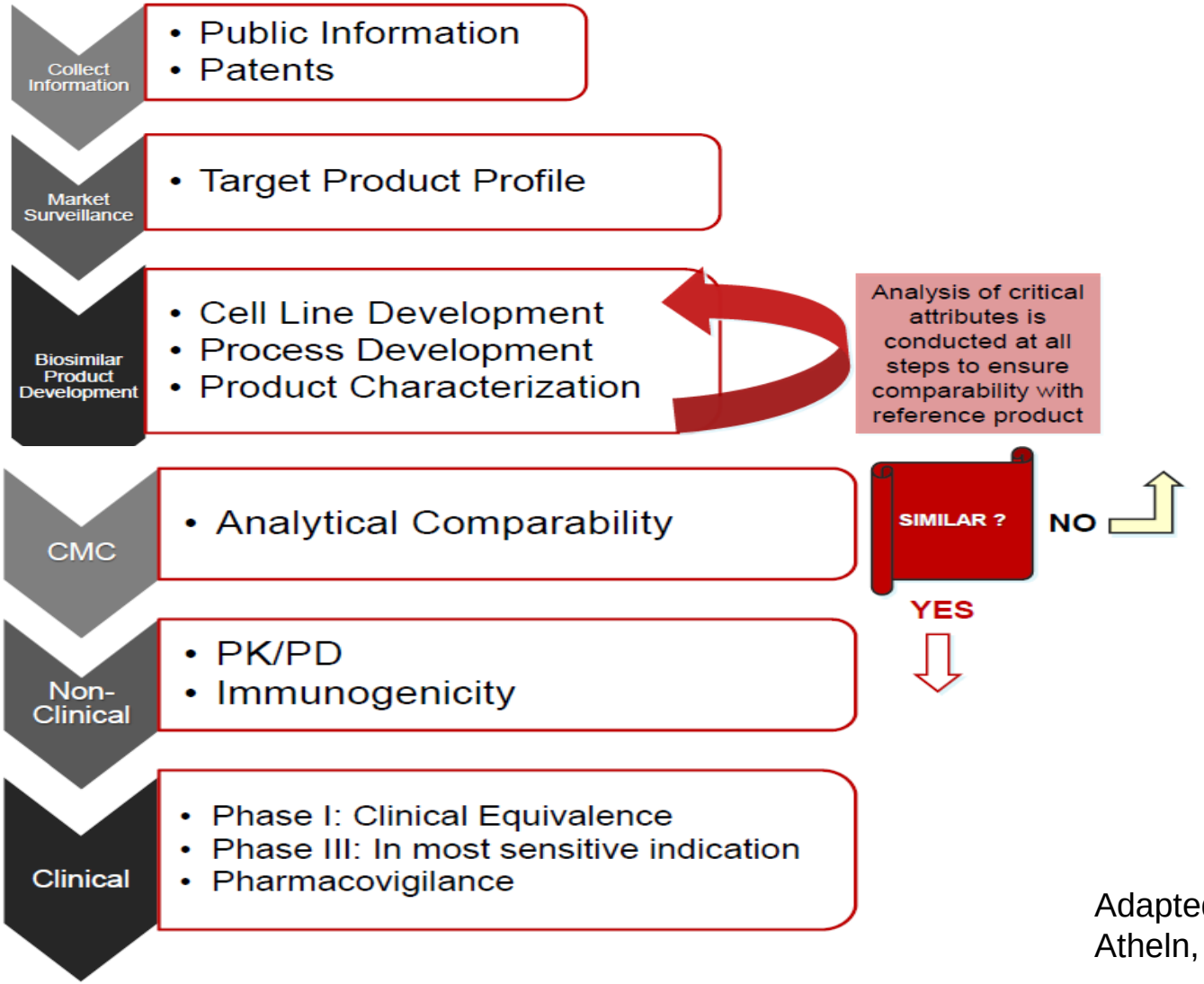
Approval for Hospira's version of J&J's Remicade has global implications

Hospira and partner Celltrion have become the first companies to gain approval in the EU for a biosimilar monoclonal antibody (mAb), winning a green light for their infliximab-based products.

The biosimilar - which will be sold as Inflectra by Hospira and Remsima by Celltrion - will compete in the EU market with the original infliximab brand **Remicade** from Johnson & Johnson.

Remicade is approved to treat rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, psoriatic arthritis and psoriasis and last year racked up European sales of over $2bn.

Roadmap to a Biosimilar



Adapted from Dr. Adriana Manzi, Atheln, Inc.

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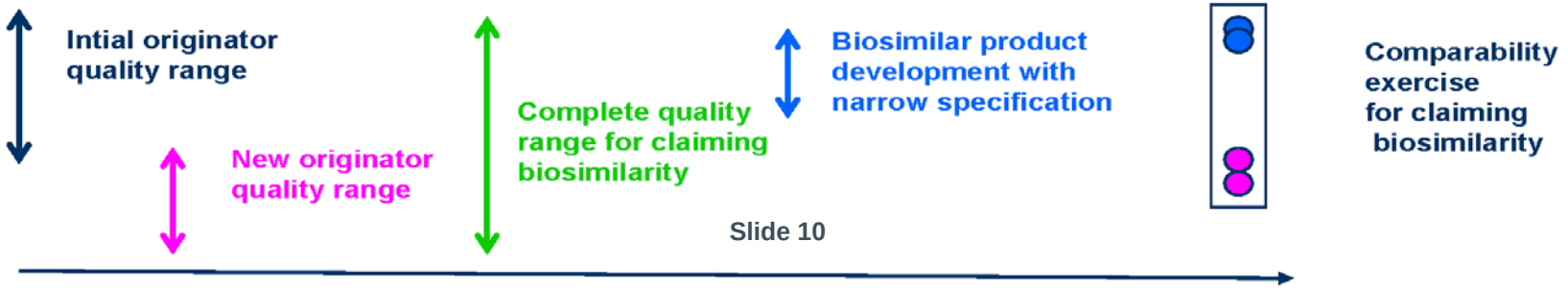
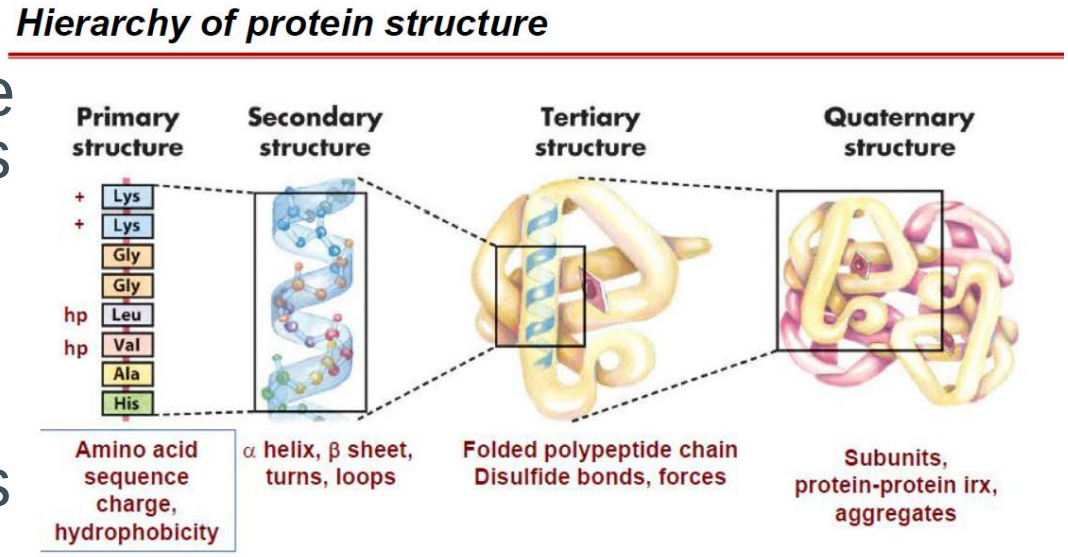
Target Product Profile

- Obtain multiple lots of Reference (Innovator) product and characterize with a set of state-of-the-art analytics, including bioassays.
- Define Critical Quality Attributes (CQA)
- Physical, chemical, biological or microbiological properties that should be within a defined range to ensure the desired product quality.
- Desired product quality = patient safety & efficacy
- Refine ranges during program as product and process understanding grows
- Set the “goal posts for each CQA by ranges found in innovator lots.



Analytical Analyses

- Side-by-side comparison to reference product in each assay is required to show comparability
- Structural assays to assess structure and secondary modifications
- Functional assays to assess target binding, cellular effects, selectivity and specificity.



Adapted from Dr. Emily Shacter, Chief, Laboratory of Biochemistry, US FDA, WCBP Biosimilar Strategy Forum, Jan 2012

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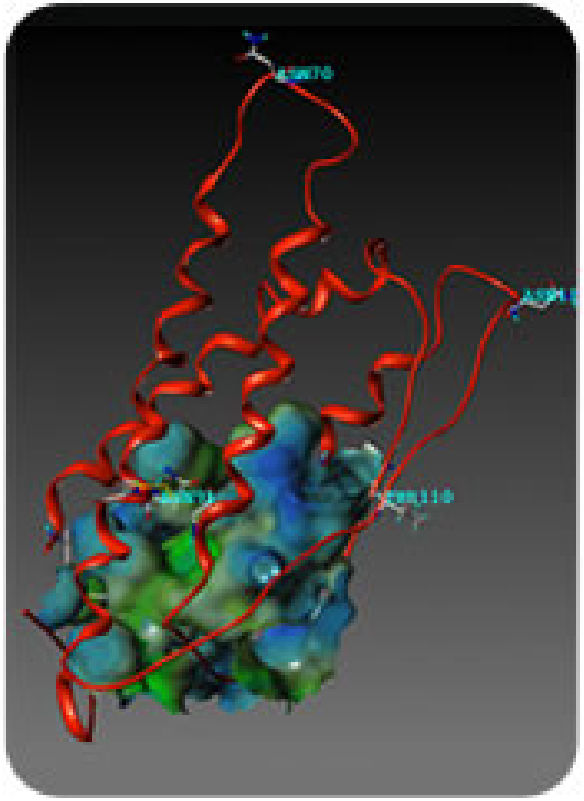
Why is Glycosylation Important?

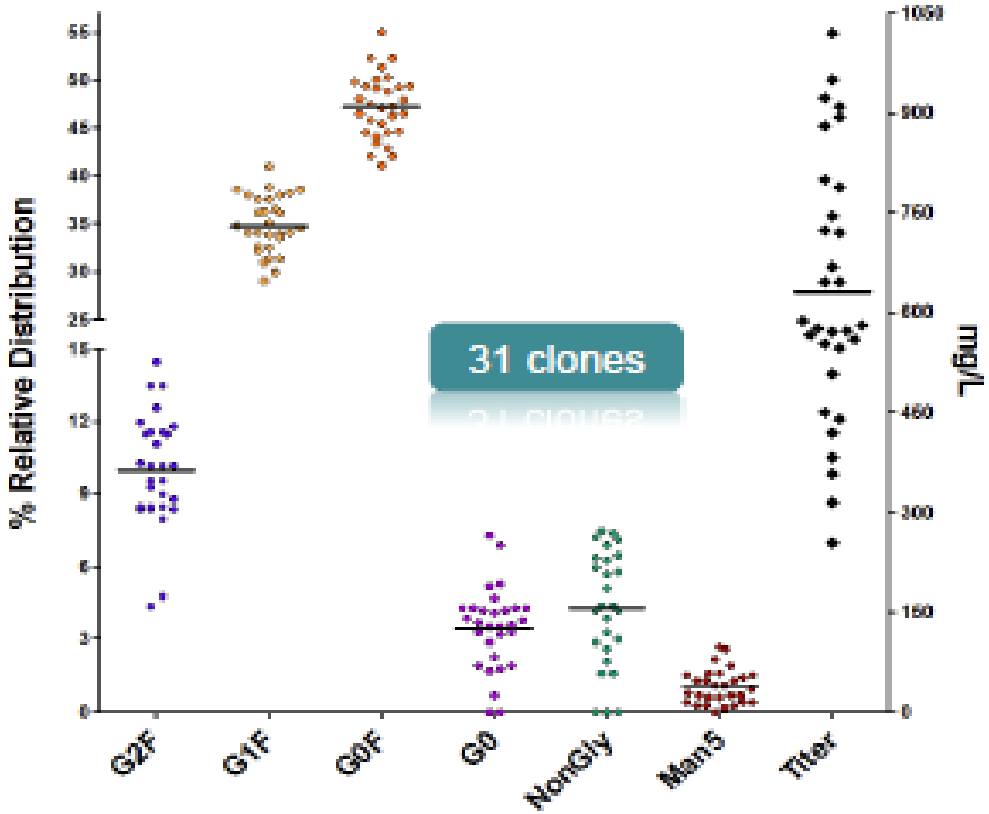
- Many complex proteins such as antibodies and enzymes are glycoproteins, containing from 2-30% carbohydrate.
- Glycosylation can affect a protein's half-life (PK) and immunogenicity in the patient, as well as binding affinity, activity and stability.
- Glycosylation is complex
- Can be attached to protein either via Asparagine (N-linked) or Serine/Threonine (O-linked)
- Multiple sugar types, each with multiple attachment sites



Structure of Interleukin-7, containing about 30% carbohydrate

Glycosylation Varies with Clone

CHOZN® GS$^{-/-}$ IgG Producing Single Cell Clones
N-Glycan Clonal Variability – FB Process



Slide courtesy of Dr. Kevin Kayser, SAFC

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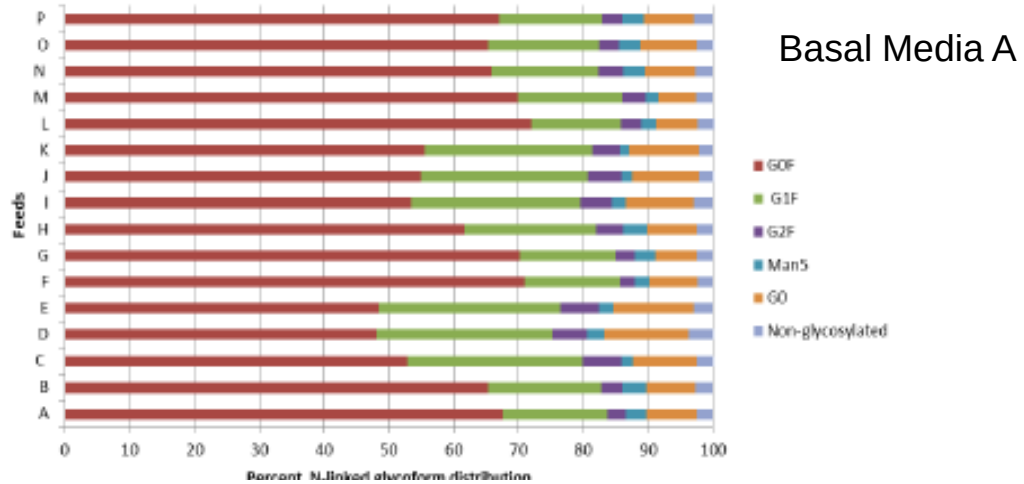
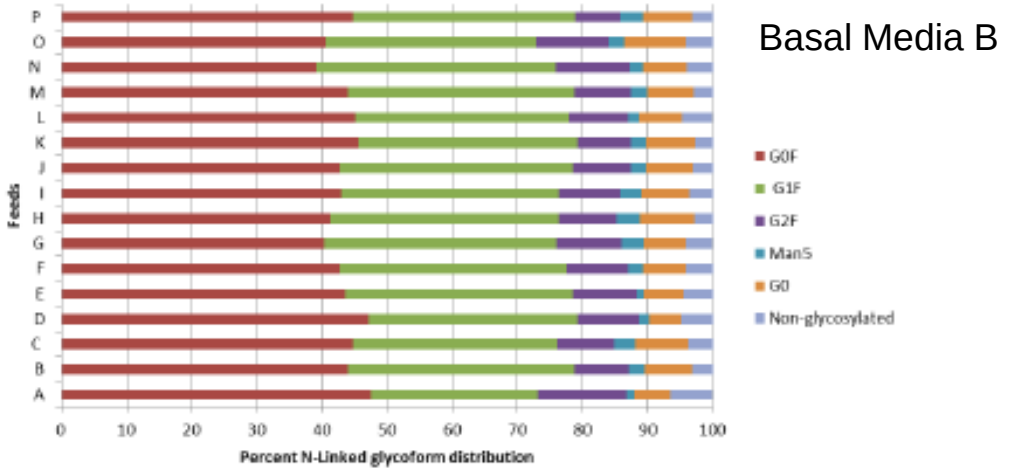
Glycosylation Varies with Basal and Feed Media

- Single Clone
- Single Mab
- Single Basal Media
  - A (top) or
  - B (bottom)
- Varied Feeds

Graphics courtesy of
Dr. Kevin Kayser,
SAFC





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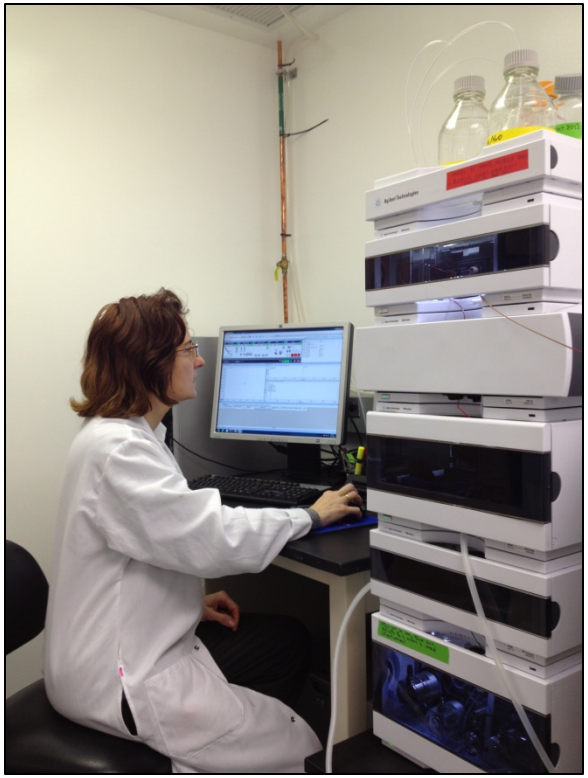
Analytical Tools to Demonstrate Comparability

Molecular Parameter	Attribute	Methods for control and characterization
Primary structure	Sum formula: Mass of light chain, heavy chain	LC-ESI-MS
	Sum formula: Mass of intact MAb	LC-ESI-MS
	Amino acid sequence	Orthogonal peptide maps with high resolution MS and MS/MS sequencing
	Disulfide bridging	Non-reducing Peptide Map
	Free cysteines	Ellman's, Peptide Map
	Thioether bridging	Peptide map, SDS-PAGE, CGE
Higher order structure	Secondary and tertiary structure	CD spectroscopy, DSC, H-D-Exchange, FT-IR
Heterogeneity: C- and N-terminal	C-terminal: ±Lys, truncation to Pro-amide	CEX with/without CBP-digest; Papain-IEX; Peptide Map, IEF
	N-terminal variants: (pGlu/Gln, pGlu/Glu)	CEX; Papain-IEX; RP-HPLC of LC, HC; Peptide Map, IEF



Analytical chemist operating Mass Spectrometer

Adapted from Dr. Adriana Manzi, Atheln, Inc., April 19, 2012

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More Analytical Tools to Demonstrate Comparability

Molecular parameter	Attribute	Methods for control and characterization
Heterogeneity: Glycosylation	Glycan isoforms: •Major (G0, G1, G2) •Minor (e.g. Unfucosylated, α-gal)	NP-HPLC of 2AB-labeled glycans, coupled to ESI-MS, exoglycosidase digestion, MALDI TOF/TOF
	Sialic Acids incl. NGNA	NP-HPLC, WAX, HPAEC; RP-HPLC after DMB-labeling
	Aglycosylated MAb	CGE, Peptide map
Heterogeneity: Glycation	Glycation of Lys	Boronate affinity; LCMS; Peptide map
Other amino acid modifications	Oxidation	RP-HPLC; Papain-HIC; Peptide map
	Deamidation	CEX; Papain-IEX; Peptide map
Heterogeneity: Size	Aggregation	SEC, FFF, MALLS, DLS, AUC; imaging methods and particle characterization
	Fragmentation at disulfides: HL, H_2L, H, L	CGE, SDS-PAGE, SEC, RP-HPLC
	Fragmentation in amino acid chain: p100, p50	CGE, SDS-PAGE, SEC, RP-HPLC

Analytical chemist operating Multi-Angle Laser Light Scattering (MALLS)

Adapted from Dr. Adriana Manzi, Atheln, Inc., April 19, 2012

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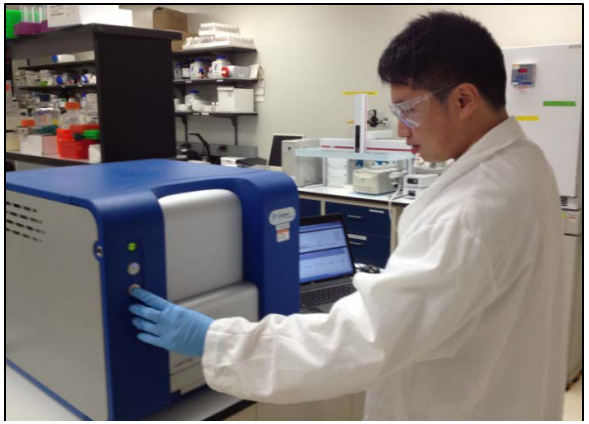
Cell-Line Development of a Biosimilar

- Genetics
- Use the same host cell or protein expression system as reference product, *i.e.*, Don't change the cell line.
- Base clone selection not only on productivity but also on product similarity to reference product
- Environmental
- Cell culture conditions, media and feeds affect both productivity and product quality.
- Analytical
- Your "eyes and ears" in this process to tell just how similar your protein is to the reference.

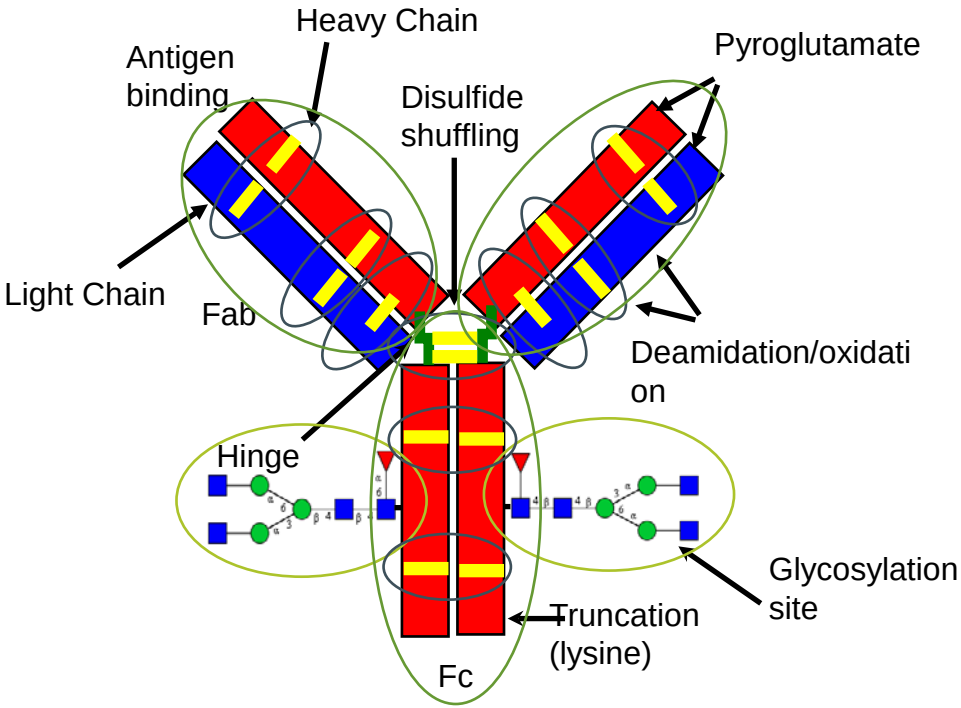
Biologist running small bioreactors



Biochemist testing product by capillary electrophoresis

Special Safety Concern for Biologics

- Immunogenicity: Ability to generate an immune response in the patient
- Potential for any biologic
- Loss of efficacy by binding up product and increasing clearance from the body
- Adverse effects possible – anaphylaxis, immune cross reaction to patient's own proteins
- Biosimilar must have a comparable molecular profile regarding potentially immunogenic areas
- Altered glycosylation
- Aggregation
- May develop changes leading to immunogenicity upon storage



IgG Antibody Structure Showing Potential Sites of Instability

(Diagram courtesy of Dr. Greg Kilby of Agilent Technologies)

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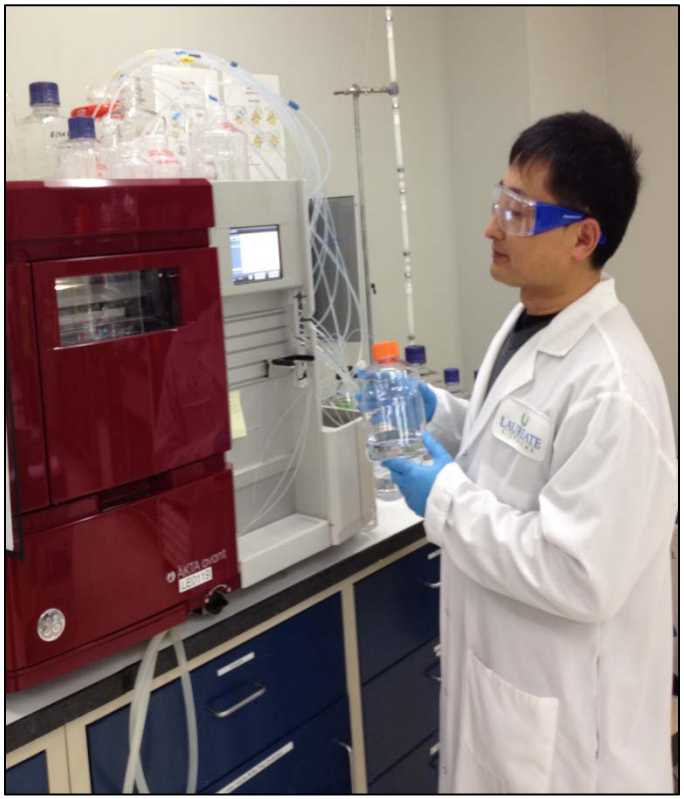
Protein Purification for Biosimilars

- Route to removal of protein variants not found in purified reference product.
- Levels of impurities such as host-cell proteins (HCP) and DNA must be comparable to or lower than reference product.
- Yield is important to cost of goods.



Purification Scientist at chromatographic workstation

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Protein Formulation

- Default is to use the same formulation as innovator
- But, new FDA Guidelines allow development of a biosimilar in a different formulation from reference product
- Potential advantage of delivery in a more convenient or stable form.
- Self-Injection device like pen
- Intranasal delivery (spray)
- Transdermal (skin patch)
- Lyophilized

Disposable Auto Injectors (Vibex™)

Pen Injectors

Reusable Needle-Free Injectors

Self-injection devices from Antares

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Scale-Up and Production

- Comparable to new biopharmaceutical programs
- Side-by-side analytical comparison of your product to reference is part of entire process

2000L Bioreactor at Gallus Biopharmaceuticals

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Biosimilars vs. Biobetters

Phase	Probability of Success (POS)		
	Novel NME	Biosimilar	Biobetter/ Next Gen
Preclinical development	86% (industry average)	95%	86%
Phase I clinical trials	84%	90%	84%
Phase II clinical trials	53%	80%**	80%**
Phase III clinical trials	74%	--	74%
Registration	96%	96%	96%
Total PTRS*	**27% from entry into preclinical development**	**65%**	**41%**

\# Using available data from multiple sources, including Reichert (2008), Paul et al. (2010), and DiMasi et al. (2010), http://www.imgt.org/IMGTmedical/Overview_of_Drug_Development.pdf, as well as other industry sources.

* PTRS, Probability of technical and regulatory success.

** Higher because target has been clinically validated.

Table from Bill Stohl, Janssen, IBC Biopharm Prod Week, 29 Feb 2012, San Diego

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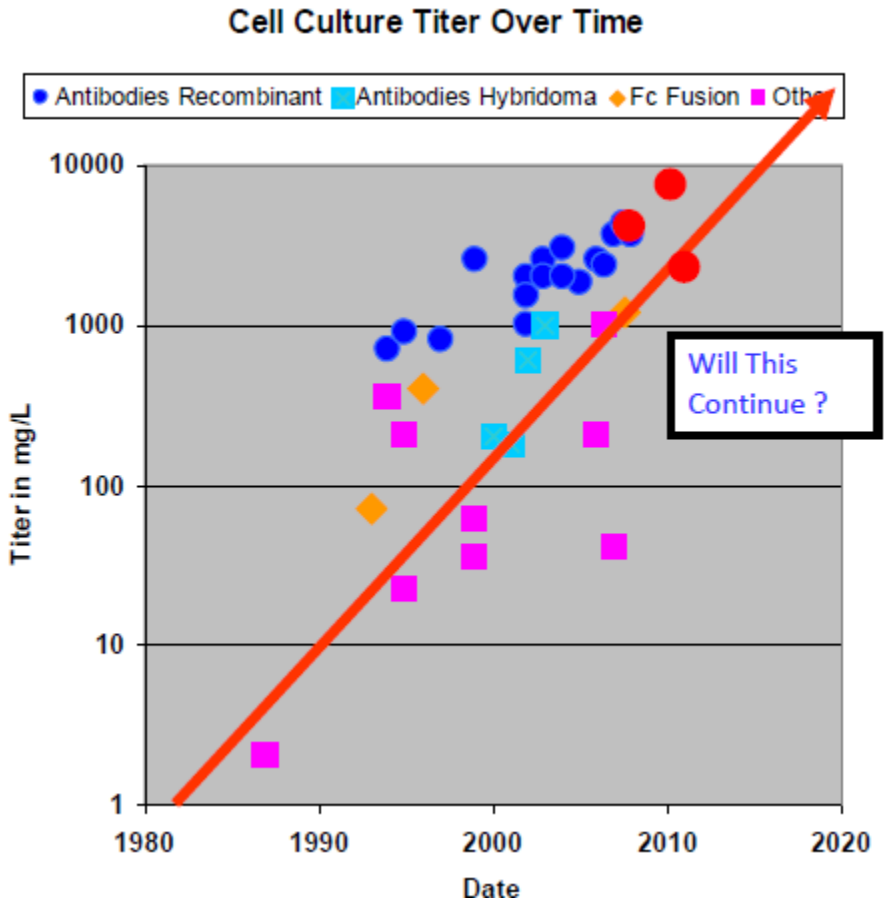
Rational for Biobetters

- Currency: Why make a copy of a drug developed 15-20 years ago, when biopharmaceutical science has advanced so far since?
- Analytics: No need to slavishly match reference product with side-by-side analytics
- Product Information: Product indications and approximate dosages are known from reference product
- Potential advantages: Longer half-life, greater activity, lower dosage, and fewer side effects.
- Cost of Goods: Potentially lower if Biobetter is made using modern high-productivity methods and reference product is not.



Graphic from Dr. Susan Jones of Bioprocess Technology Consultants

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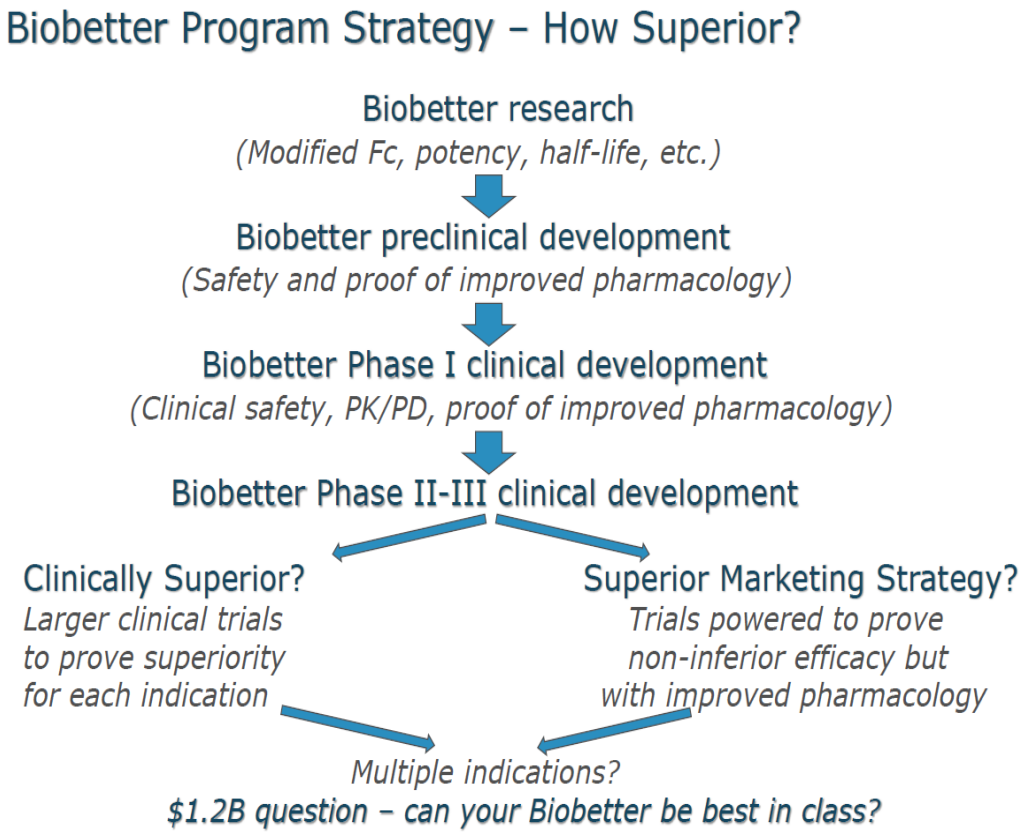
Drawbacks to Biobetters

- Longer and more expensive clinical trials – must follow new-drug approval pathway
- How much better? Must be significantly better to gain acceptance over established reference product or biosimilars thereof.
- Large clinical trials likely needed to prove clinical superiority over reference product



Graphic from Bill Stohl, Janssen, IBC Biopharm Prod Week, 29 Feb 2012, San Diego

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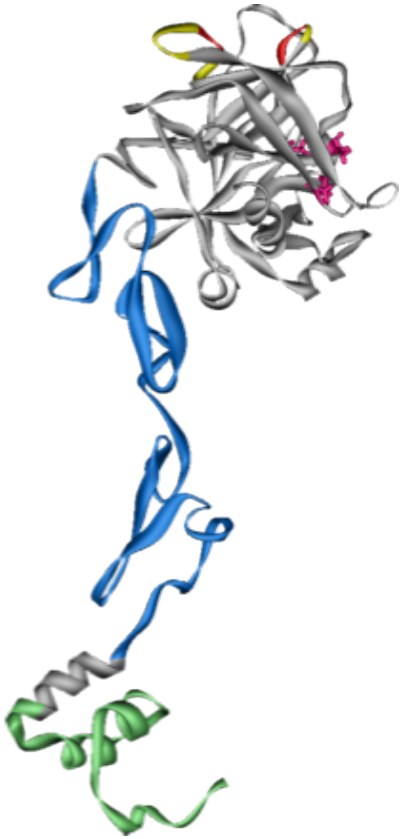
Example of Biobetter of Protein C

- Protein C is produced in the liver and upon activation becomes a serine protease with multiple activities
- Anti-coagulant
- Anti-inflammatory
- Cytoprotective
- Recombinant activated Protein C was sold for ten years until it was removed from the market in Oct 2011
- ZZ Biotech re-engineered the Protein C structure to reduce markedly the anticoagulant activity that was a major deterrent to use of Protein C in therapy of stroke and other neurological disorders.
- Gallus (as Laureate Biopharma) developed and produced this variant form for ZZ Biotech for Phase 1 trials, which were recently completed.



3K3A-APC Variant of Protein C

Graphic courtesy of ZZ Biotech

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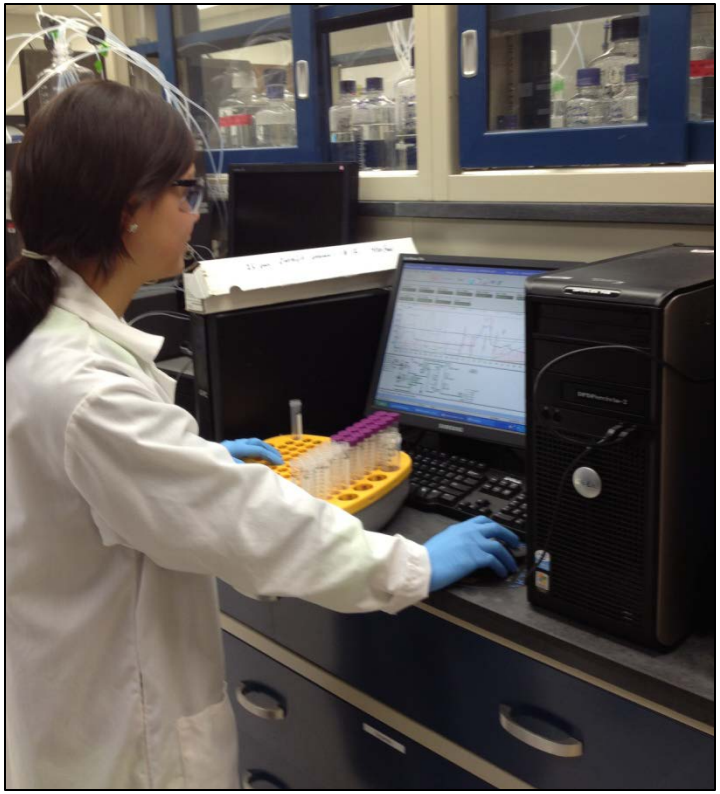
Conclusions & Recommendations

- Biosimilars allow broader patient access and lower-cost biopharmaceuticals
- Strong analytical techniques are essential to prove biosimilarity from the earliest stages of development
- Biobetters offer a route to an improved product but must be significantly better to be accepted.
- Recommend pre-IND meetings with FDA to review your strategy and development program



Biochemist developing purification process

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For Additional Reading

- *FDA Guidances for Industry, Quality & Scientific Considerations in Demonstrating Biosimilarity to a Reference Protein Product*, www.fda.gov
- *European Guidance Documents on Biosimilars*, www.ema.europa.eu/ema
- *Elucidating Biosimilars Characterization*, Biopharm Int, Sept 2013, 20-31
- *Biosimilars for the Real World*, Bioproc Intl, June 2013 Supplement.
- *Biobetters May Prove a Better Bet*, Chem & Eng News, 91(12) 24-25 (Mar 2013).
- Information on Gallus Biopharmaceuticals, LLC, www.gallusbiopharma.com

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