Biologicals and biosimilars – scientific aspects of production and quality control

Univ. Prof. Dr. Harald H. Sitte

Institute of Pharmacology

Medical University of Vienna

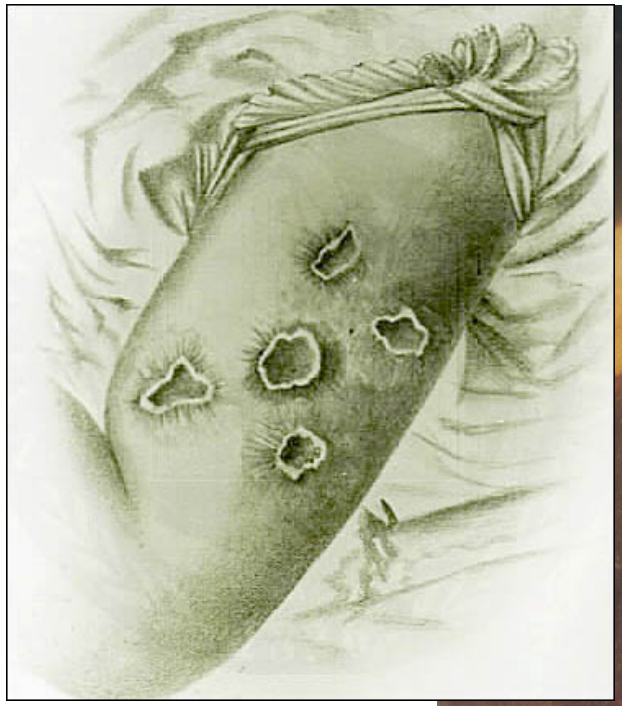
Financial disclosure (past 5 years)

Lectures:
AbbVie, Aesca, Amgen, Astellas, AstraZeneca, Astropharma, IIR, MSD, Mundipharma, Pfizer, Ratiopharm, Roche, Sandoz, Shire

Consulting:
AbbVie, Amgen, Chiesi, Gebro, Janssen-Cilag, Lundbeck, Serumwerk Bernburg, Shire

Membership of advisory boards:
Amgen, Roche, Sanofi-Aventis

Dr. Edward Jenner vaccinates James Phipps



What are *'Biologicals'*?

- *Vaccines (Toxins/Toxoids)*
- *Blood products*
- *Cytokines and related*
- *Hormones*
- *Monoclonal Antibodies*

➔ ***Compounds, made by bioorganisms (nowadays recombinant)***

What are *'Biolosimilars'*?

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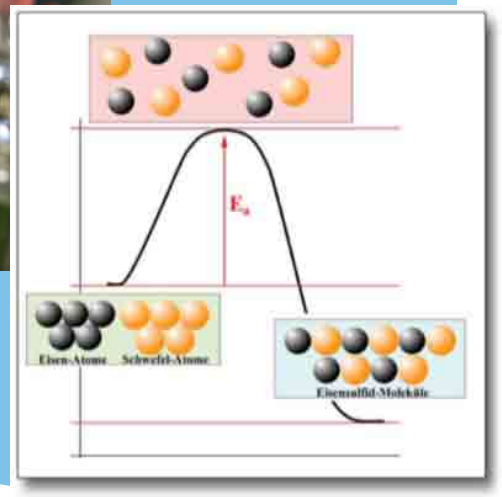
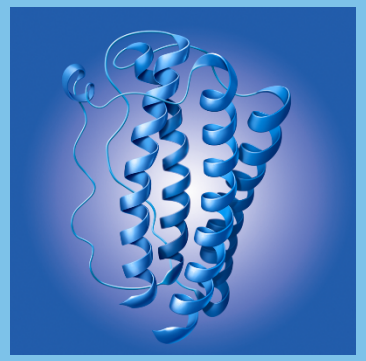
➔ ***Compounds, made by bioorganisms (nowadays recombinant)***

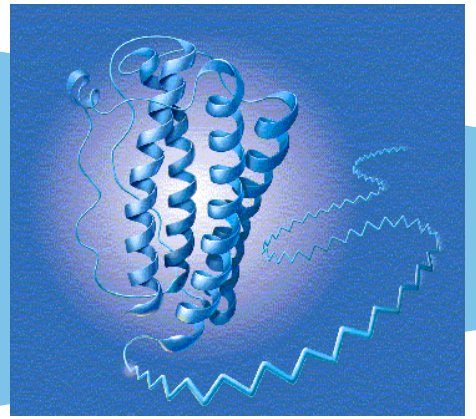
Acetylsalicylic Acid



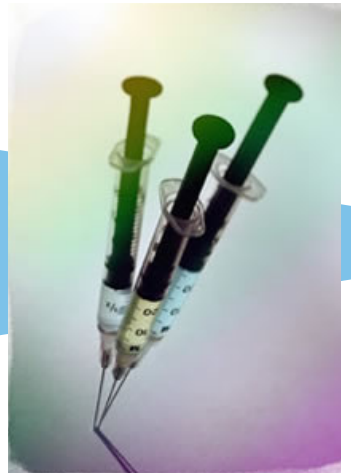
www.chemischereaktionen.de/einf02.html



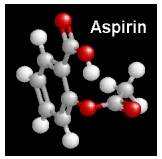
G-CSF = Filgrastim



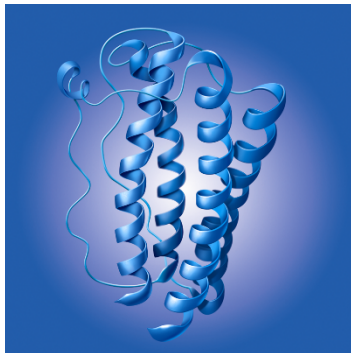
PEG-Filgrastim



Receptor theory



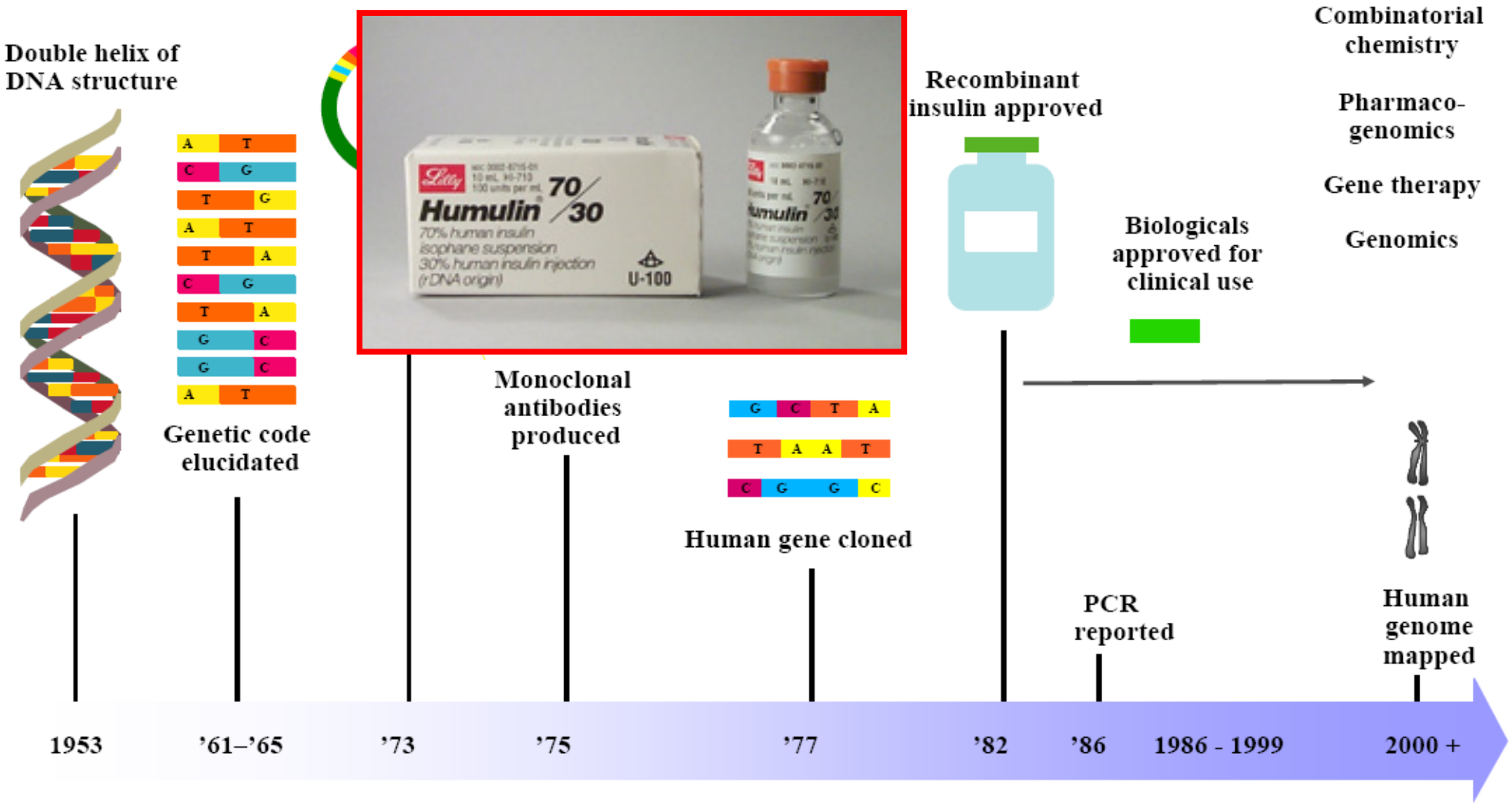
"Ligand"



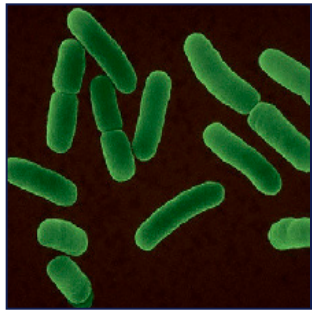
"Ligand"

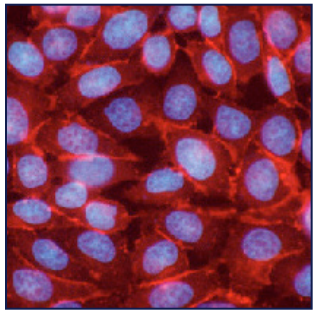
1878 Langley – Ehrlich 1909

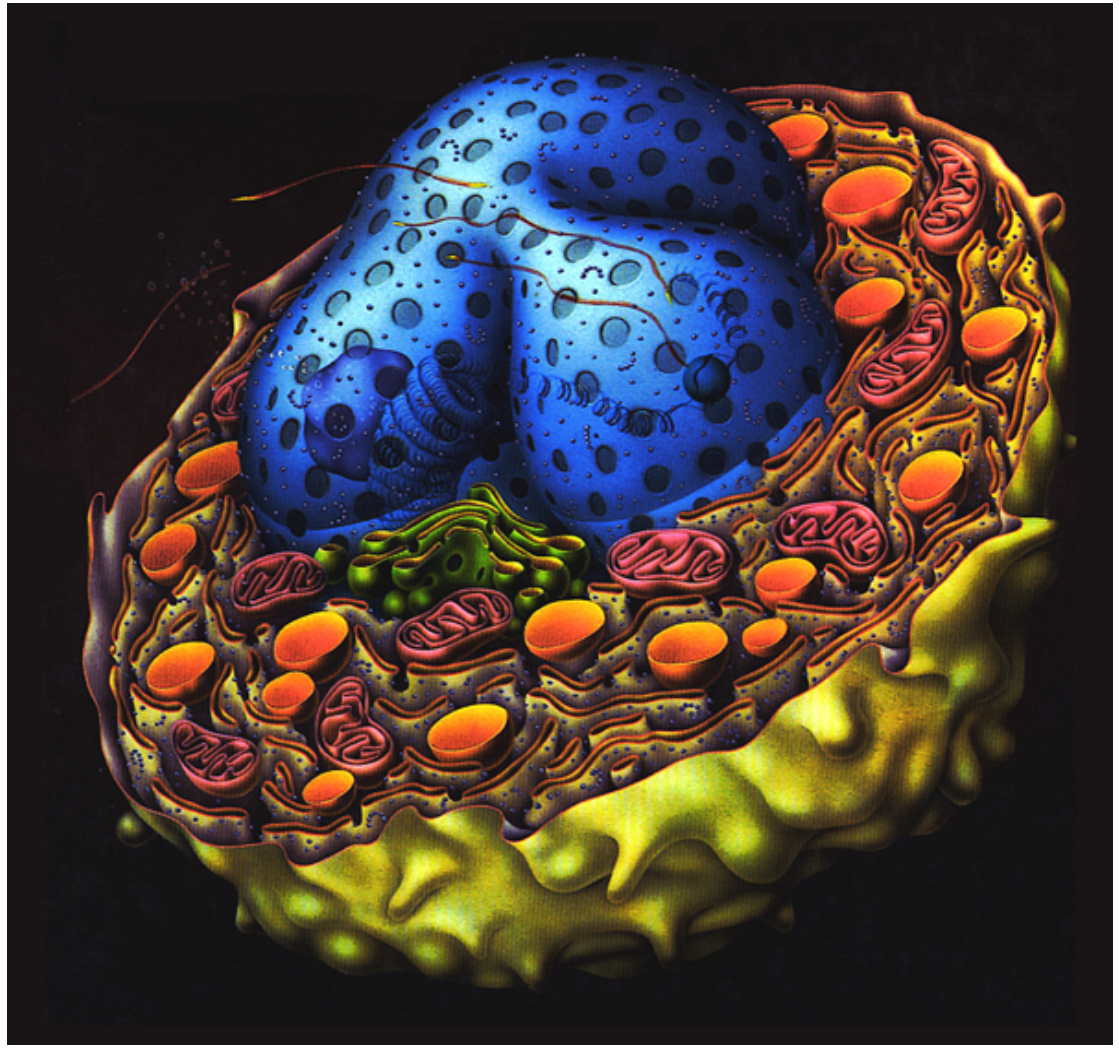
Biotechnology – a success story



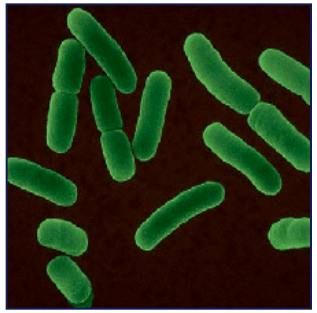
From genetic code to protein

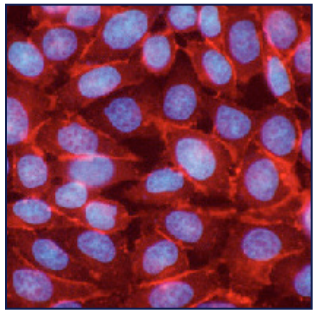


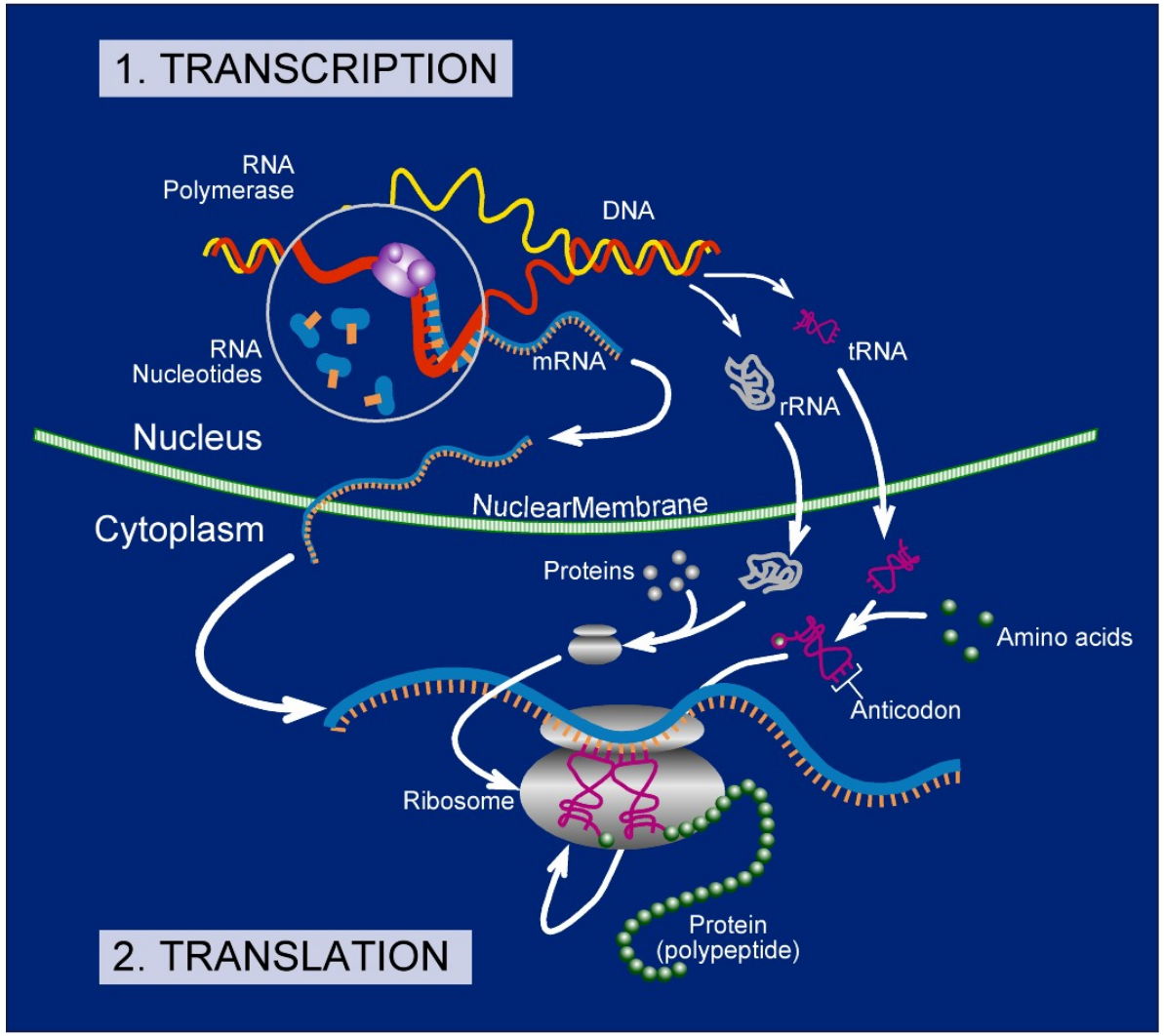




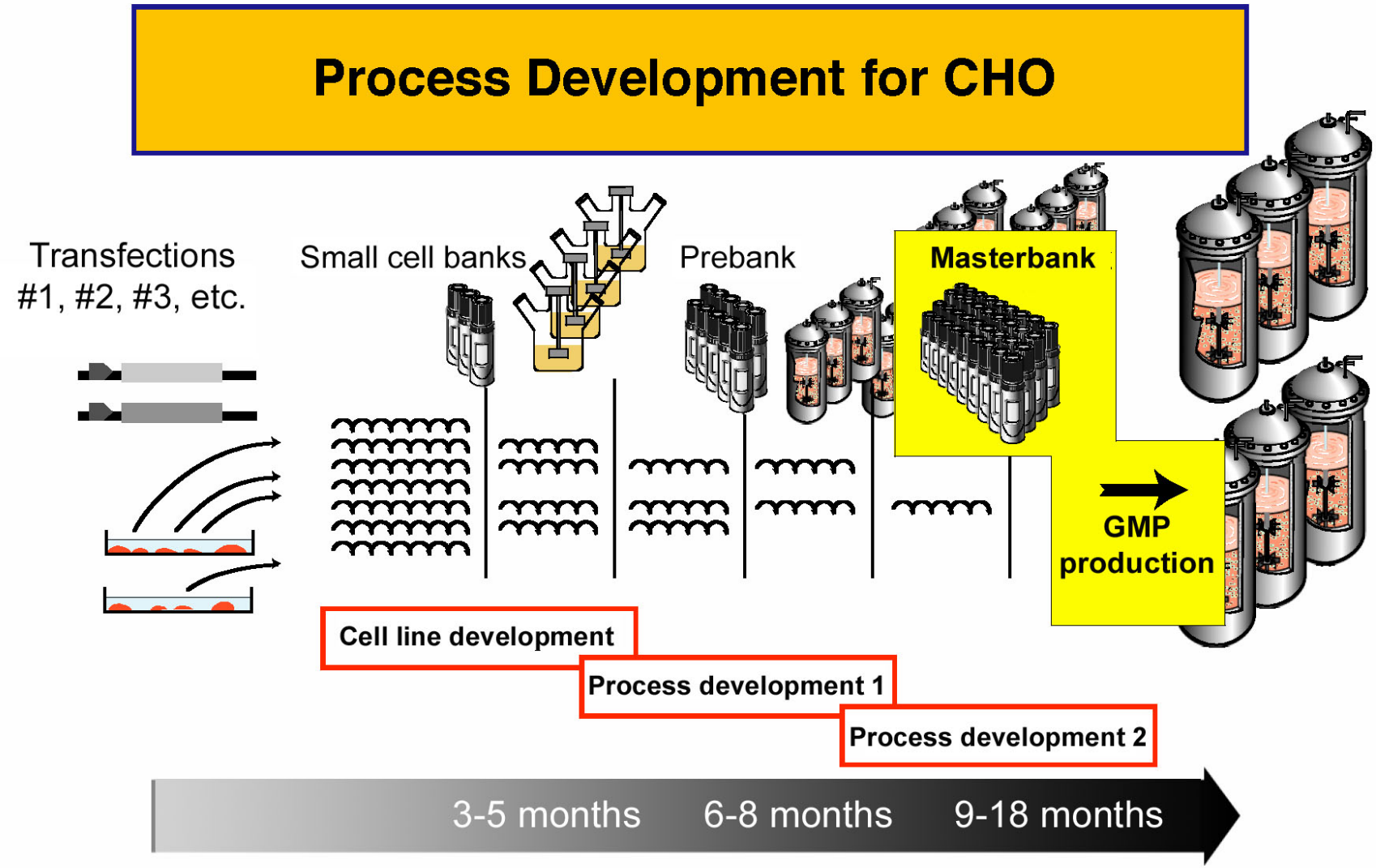
From genetic code to protein







Mass production of pharmaceutical products



The purity of biological drugs: gel electrophoresis during the manufatucring process

Manufacturing Process

Bacterial expression

Includion bodies collection

Solubilization

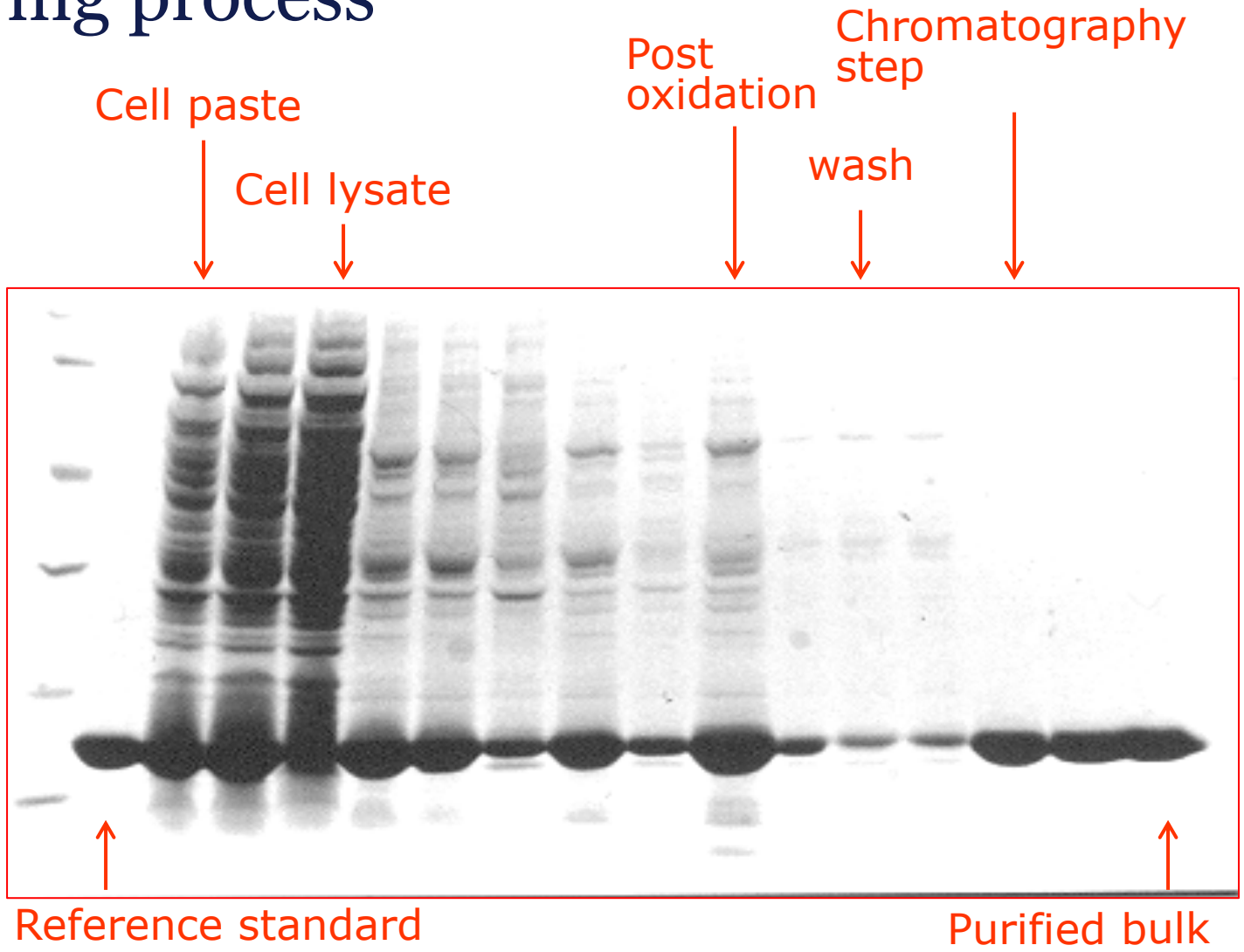
Oxidative folding

Chromatographies

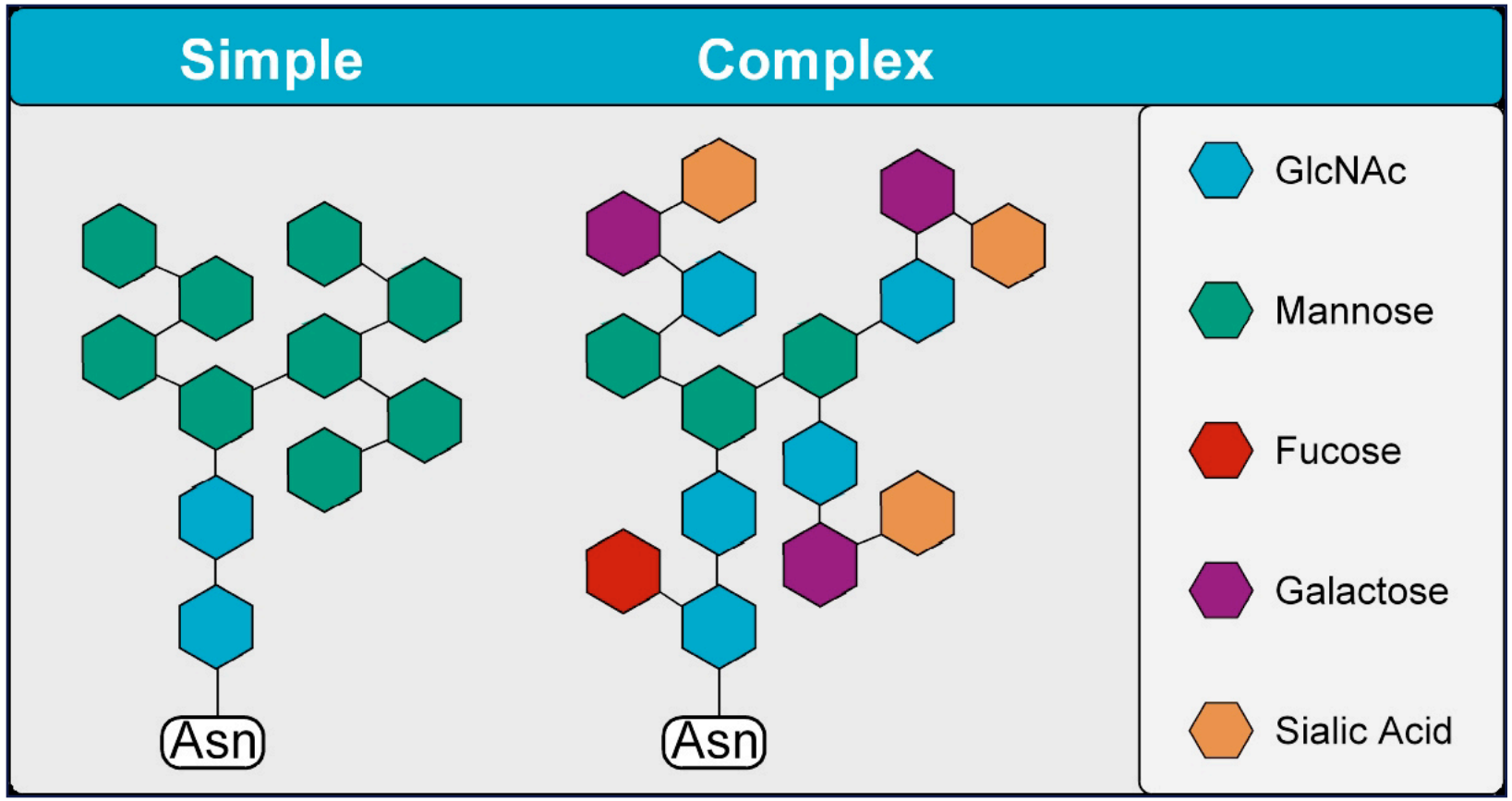
Filtration/ concentration

Purified bulk

Formulation



Glycosylation: dependent on the host cell



Importance of glycosylation

- Biological activity
- Ligand recognition
- Ligand binding
- Antigenicity
- Folding
- Trafficking/Targeting
- Clearance
- Stability
- Immunogenicity

Importance of glycosylation

Biochemistry **1992,** *31,* 9871–9876

Articles

Role of Glycosylation on the Secretion and Biological Activity of Erythropoietin

Evelyne Delorme,* Tony Lorenzini, James Giffin, Frank Martin, Frederick Jacobsen, Tom Boone, and Steve Elliott

Amgen Inc., Amgen Center, Thousand Oaks, California 91320

Received April 3, 1992; Revised Manuscript Received July 21, 1992

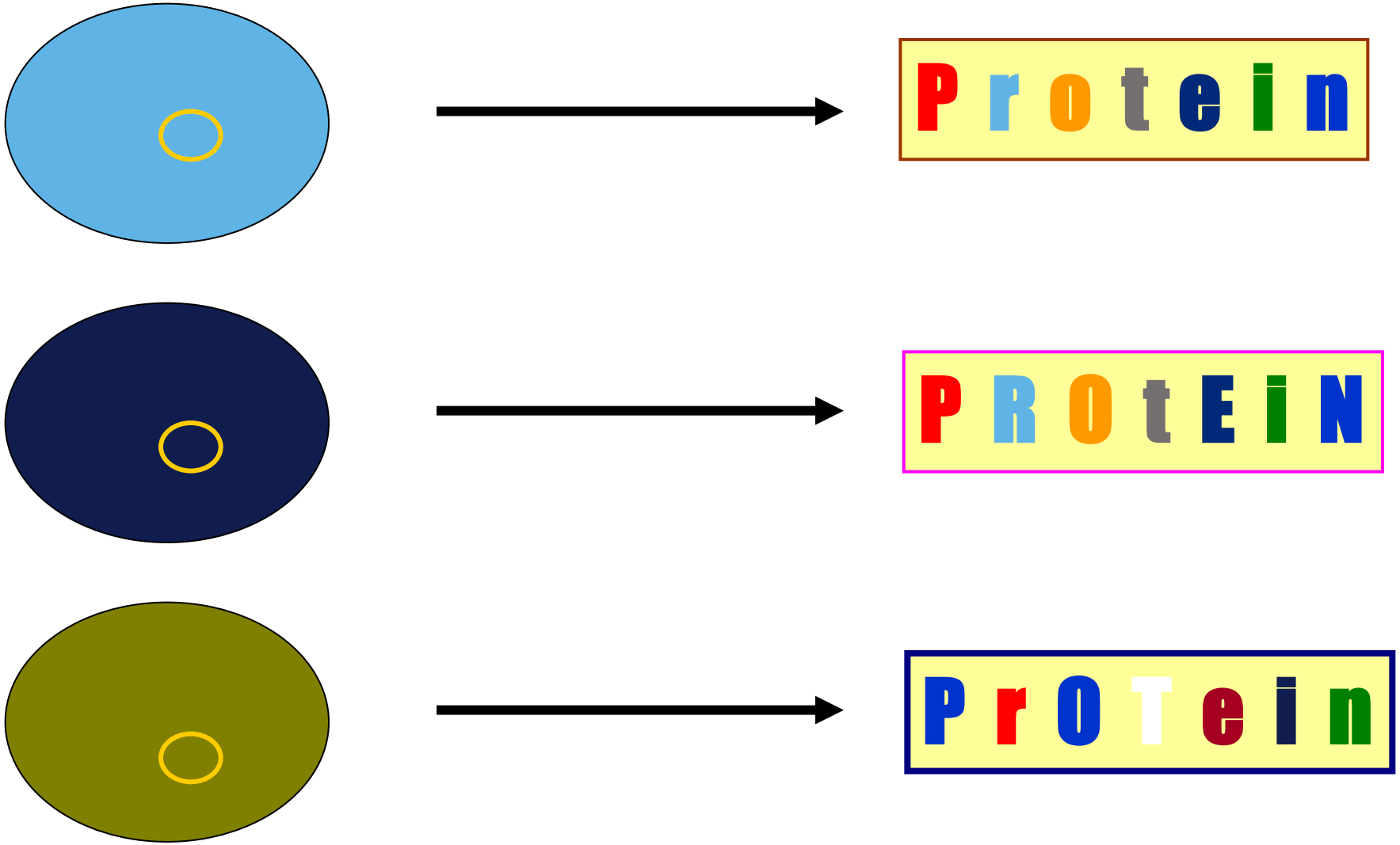
ABSTRACT: The erythropoietin (EPO) molecule contains four carbohydrate chains. Three contain N-linkages to asparagines at positions 24, 38, and 83, and one contains an O-linkage to a serine at position 126. We constructed human EPO variants that eliminated the three N-glycosylation sites by replacing the asparagines with glutamines singly or in combinatic serine with glutamine, valine, histidin another with a triple mutation (Gln24,3 RNA encoding these variants was prese were secreted normally. Removal of an biological activity of the EPO molecule. normally. In vitro activity was also unal variant was tested in vivo, and its specif indicates that the O-linked carbohydra

The Importance of N- and O-Linked Oligosaccharides for the Biosynthesis and In Vitro and In Vivo Biologic Activities of Erythropoietin

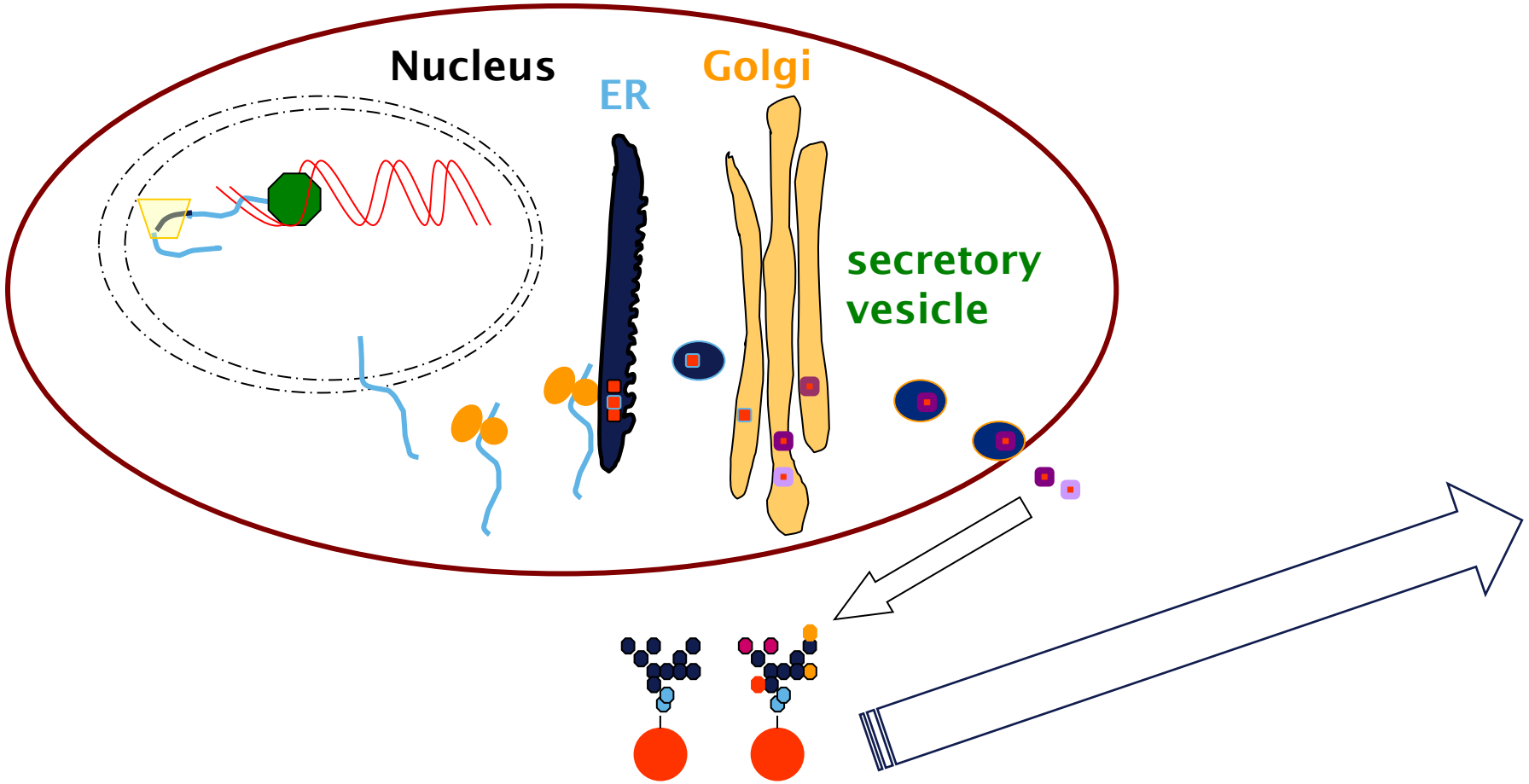
By Louise C. Wasley, Gregg Timony, Patricia Murtha, John Stoudemire, Andrew J. Dorner, Jaime Caro, Monty Krieger, and Randal J. Kaufman

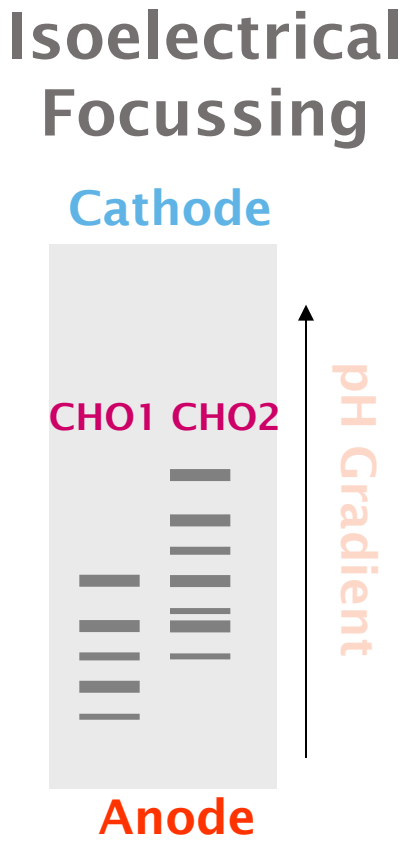
Erythropoietin (EPO) plays a critical role in stimulating the proliferation and differentiation of erythroid precursor cells. EPO is heavily glycosylated with three asparagine (N)-linked tetraantennary oligosaccharides that may contain N-acetyllactosamine repeats and a single serine (O)-linked oligosaccharide. EPO expressed in Chinese hamster ovary cells exhibits biologic properties and amino acid and carbohydrate composition similar to natural urinary EPO. The importance of the complex N-linked and the O-linked carbohydrate was studied by expressing EPO in cells that are deficient in UDP-galactose/UDP–N-acetylgalactosamine 4-epimerase activity. In these cells, the ability to add galactose and N-acetylgalactosamine to glycoproteins can be controlled by the addition of these sugars to the culture medium. The results demonstrate that a block in O-linked glycosylation and/or the ability to process N-linked carbohydrate to completion does not alter EPO secretion. EPO produced without O-linked carbohydrate exhibits normal in vitro and in vivo biologic activity and in vivo clearance. However, EPO produced with incompletely processed N-linked oligosaccharides exhibits normal in vitro activity but is at least 500-fold less effective in stimulating erythropoiesis in vivo. Studies on the survival of bioactive EPO remaining in the circulation demonstrated that EPO with incomplete N-linked oligosaccharides exhibits a sevenfold increased rate of clearance. However, this increased clearance may not fully account for the 500-fold loss of in vivo activity. These results suggest a potentially important unique requirement for appropriate complex N-linked oligosaccharides for the intrinsic biologic activity of EPO in vivo.

Differences between biologics

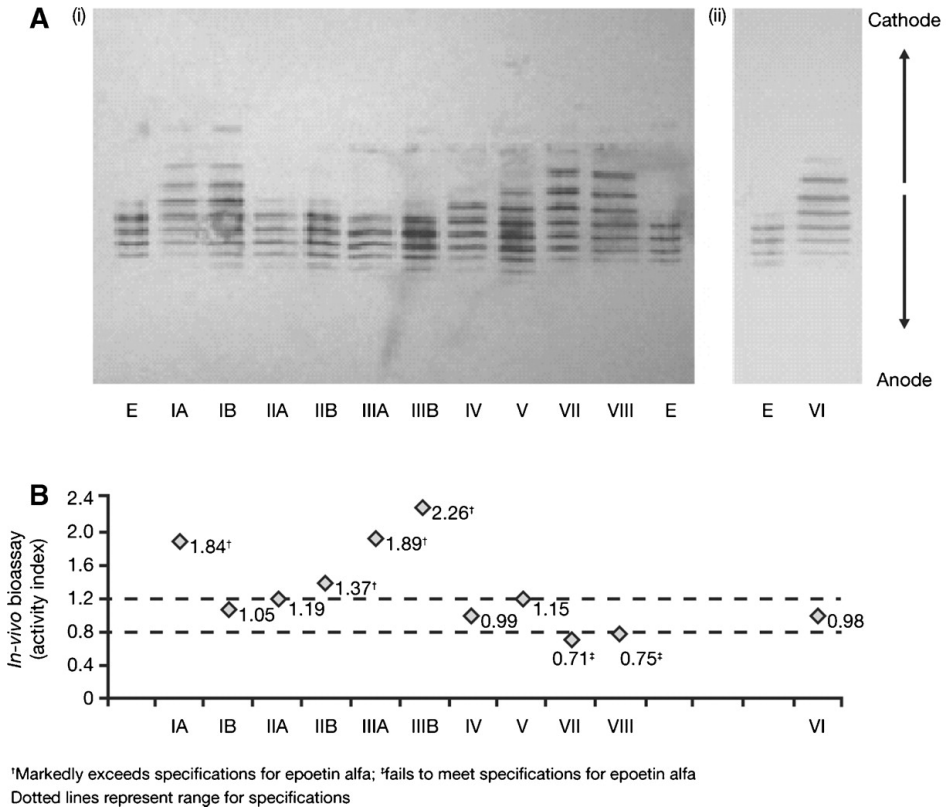


Heterogeneity of proteins by inhomogeneous glycosilation





Heterogeneity of proteins by inhomogeneous glycosilation

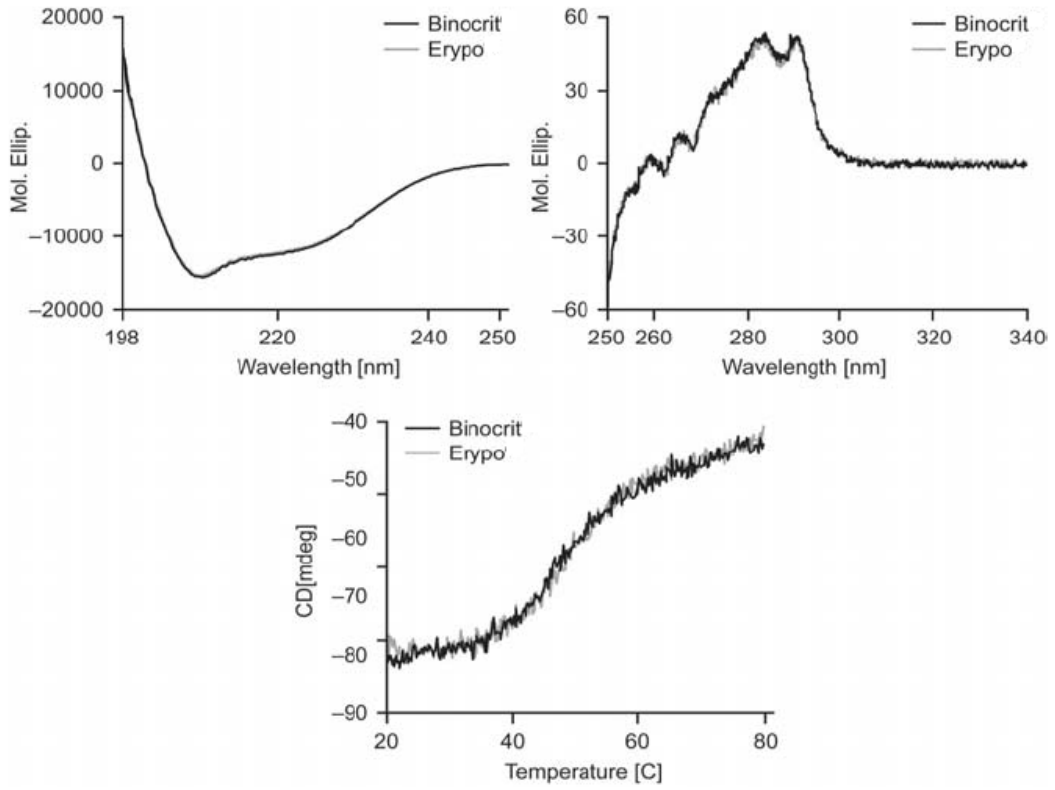


Sample	Concentration (IU/mL)	Country
IA	2000	Korea
IB	4000	Korea
IIA	2000	Korea
IIB	10 000	Korea
IIIA	2000	Korea
IIIB	10 000	Korea
IV	2000	Argentina
V	10 000	Argentina
VI	4000	India
VII	10 000	China
VIII	6000	China

. (**A**) Isoelectric focusing (i)/ western blot (ii) isoform distribution of 11 non-innovator epoetins compared to Eprex® (E). The table shows the location where each sample was obtained. (**B**) Bioactivity, determined by an *in vivo* bioassay in mice, was higher than specifications in four samples (137–226%) and below specifications in two samples (71–75%) [35].

A direct comparison: Erythropoetin alfa

Figure 3: Comparison of far- (top left) and near-ultraviolet circular dichroism -spectra (top right) of Binocrit and epoetin alfa



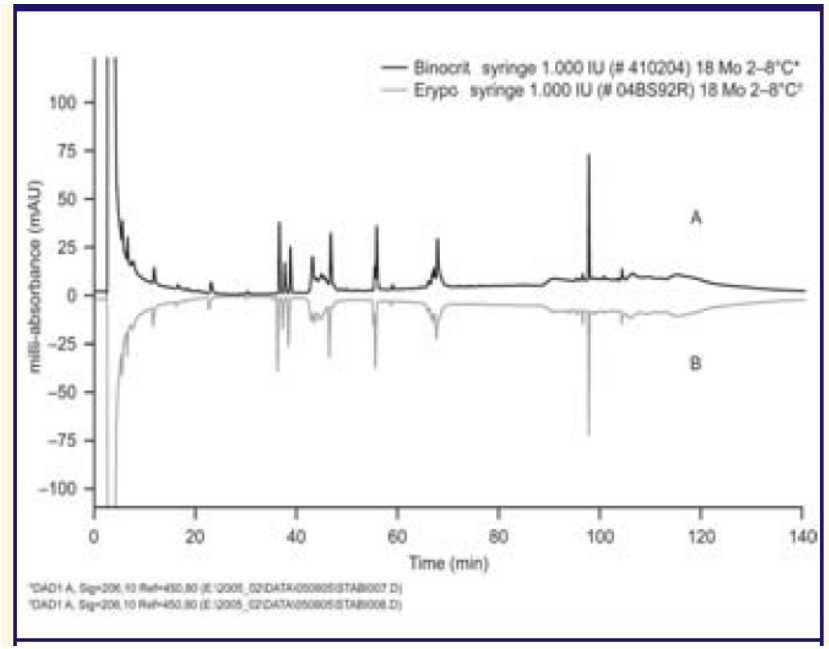
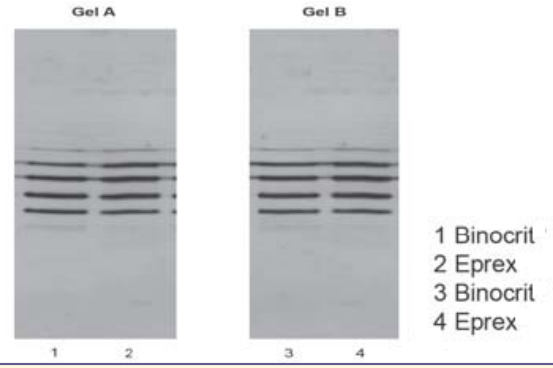
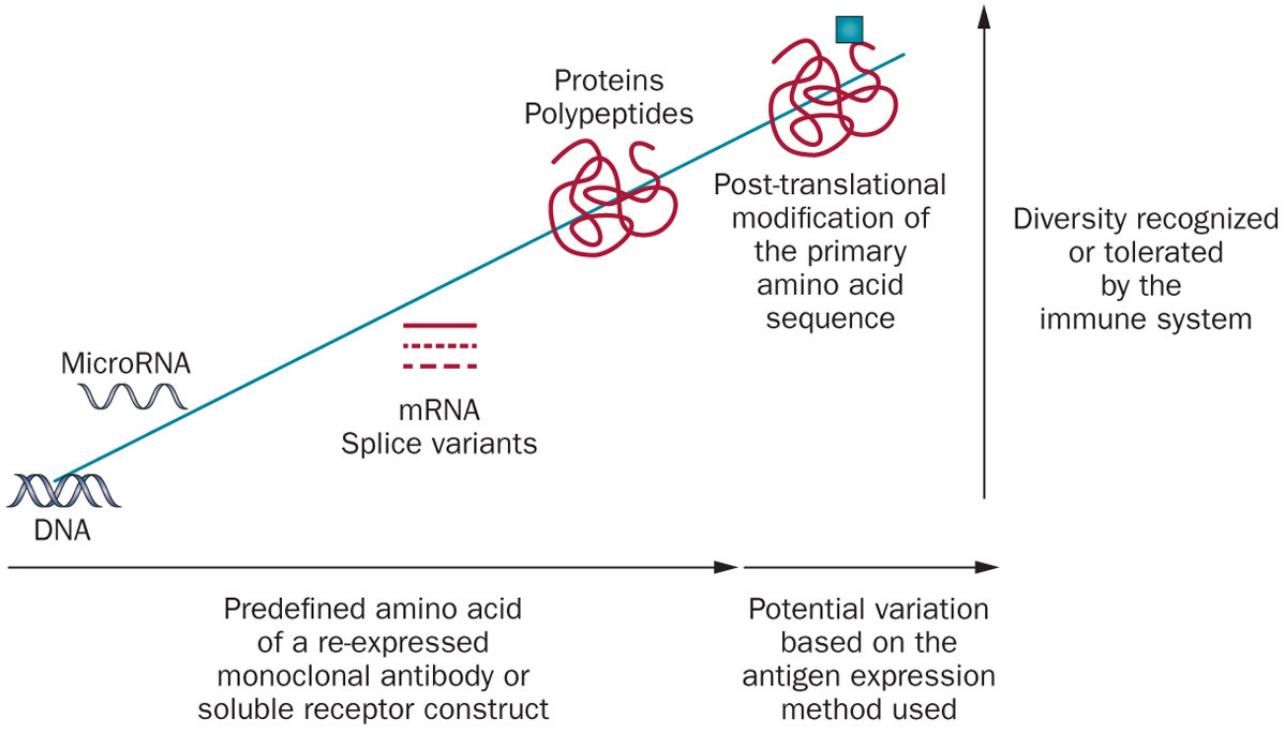
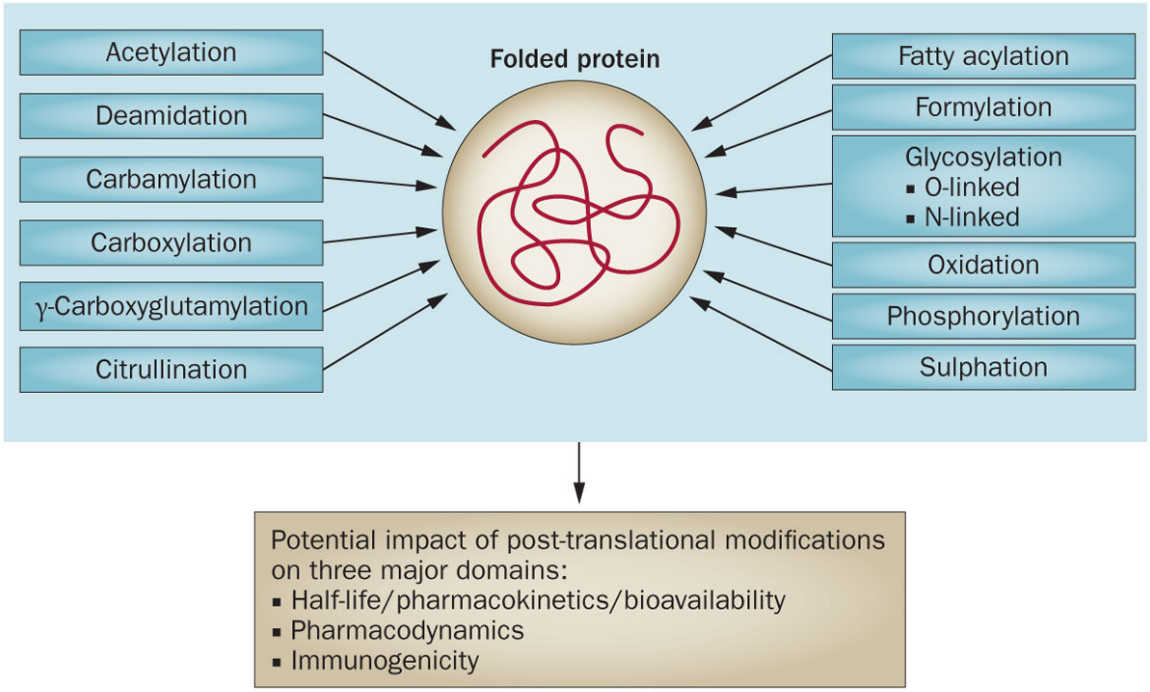


Figure 4b: Comparison of the isoform pattern for Binocrit and comparator product epoetin alfa by isoelectric focusing gel electrophoresis



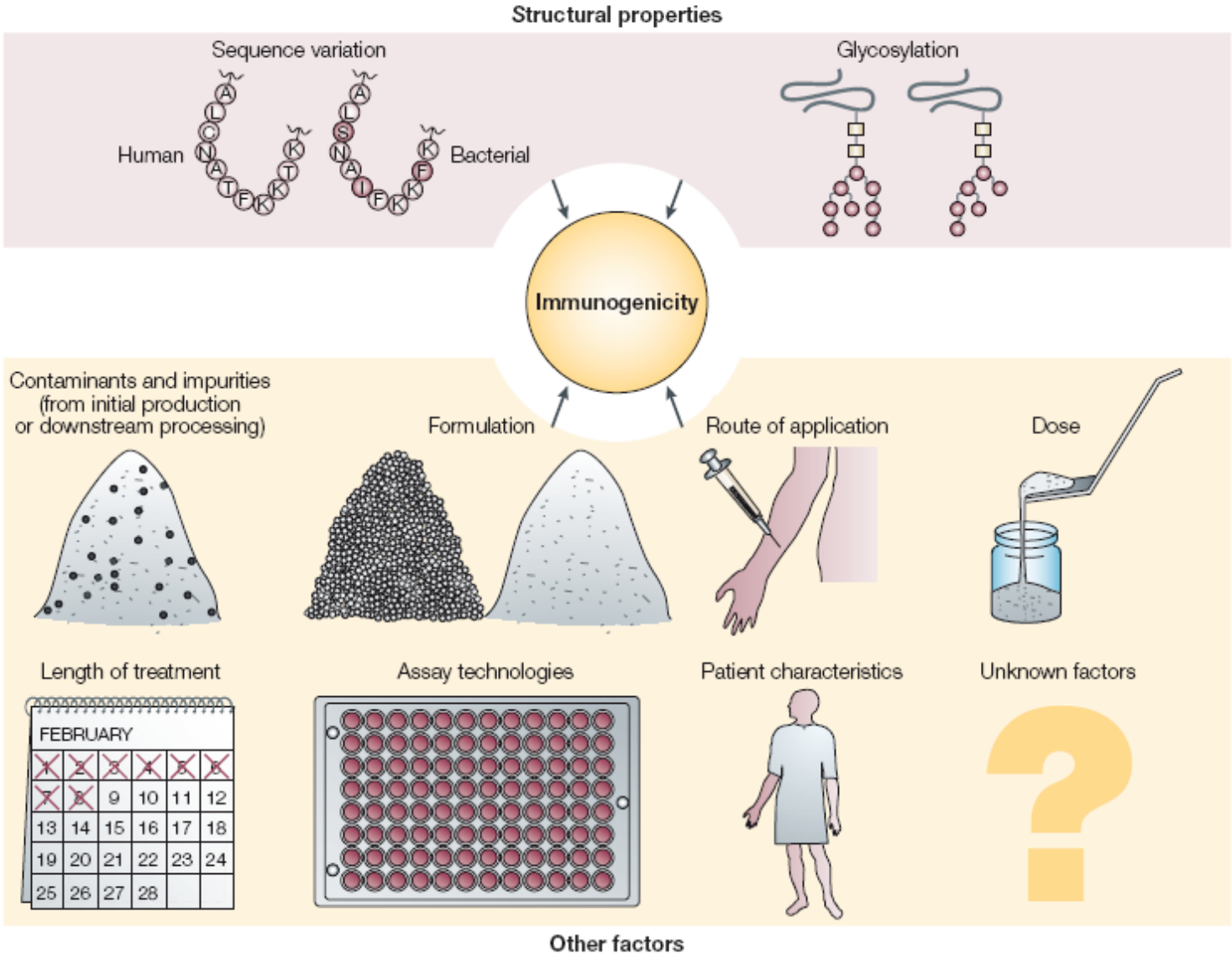
Mechanisms of protein diversity and consequences for the immune system





Nature Reviews | Rheumatology

Immunogenicity?



Possib

Potentia
efficacy

Insulin

Streptokin

Staphyloki

ADA

Salmon ca

Factor VIII

Interferon

Interferon

IL-2

GnRH

TNFR55/Ig

HCG

The NEW ENGLAND JOURNAL of MEDICINE

s”

ORIGINAL ARTICLE

Pure Red-Cell Aplasia and Epoetin Therapy

Charles L. Bennett, M.D., Ph.D., M.P.P., Stefano Luminari, M.D., Allen R. Nissenson, M.D., Martin S. Tallman, M.D., Stephen A. Klinge, B.A., Norene McWilliams, J.D., M.P.H., June M. McKoy, M.D., J.D., M.P.H., Benjamin Kim, M.D., E. Allison Lyons, B.A., Steve M. Trifilio, R.P.H., Dennis W. Raisch, Ph.D., Andrew M. Evens, D.O., Timothy M. Kuzel, M.D., Glen T. Schumock, Pharm.D., M.B.A., Steven M. Belknap, M.D., Francesco Locatelli, M.D., Jerôme Rossert, M.D., Ph.D., and Nicole Casadevall, M.D.

ABSTRACT

BACKGROUND

Between 1988 and 1998, antibody-associated pure red-cell aplasia was reported in three patients who had undergone treatment with recombinant human erythropoietin (epoetin). Between 1998 and 2000, 13 such cases were reported from France — 12 in patients who had received the Eprex formulation of epoetin alfa and 1 in a patient who had received Neorecormon (a formulation of epoetin beta); both are products that are marketed outside the United States.

METHODS

From the Midwe
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cology (C.L.B., M
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of General Interr
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Institute for Heal
Policy Studies (C.
Northwestern Ur

What are Biosimilars?
Delineation against '*non-innovator copies*' & "*stand-alone applications*"

Table 1 Proposal for a more precise terminology.

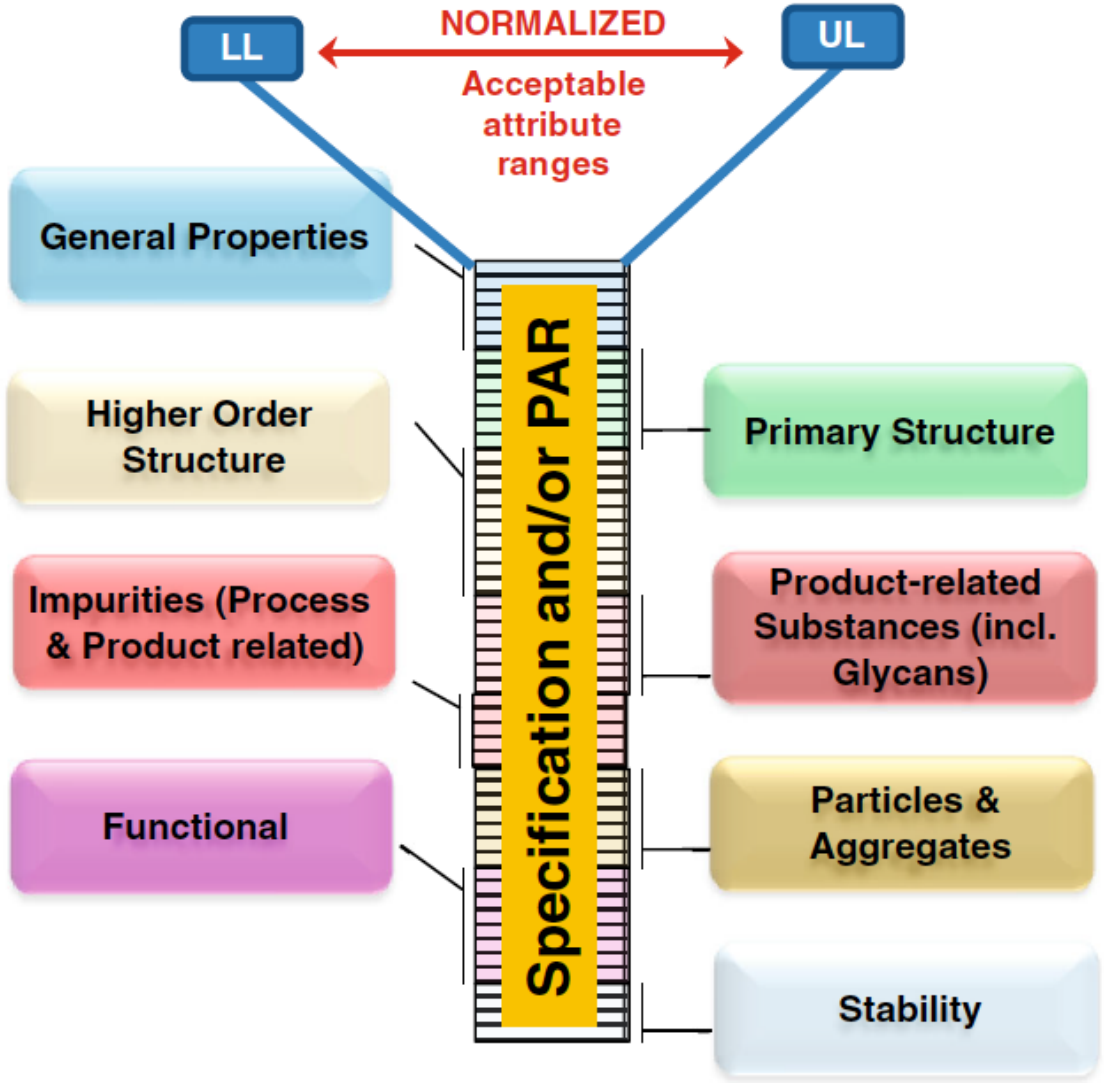
Term(s)	Definition	Implications
Biosimilar[a]	Copy version of an already authorized biological medicinal product with demonstrated similarity in physicochemical characteristics, efficacy and safety, based on a comprehensive comparability exercise.	Only very small differences between biosimilar and reference with reassurance that these are of no clinical relevance. Extrapolation of clinical indications acceptable if scientifically justified.
Me-too biological/biologic Noninnovator biological/biologic	Biological medicinal product developed on its own and not directly compared and analyzed against a licensed reference biological. May or may not have been compared clinically.	Unknown whether and which physicochemical differences exist compared to other biologicals of the same product class. Clinical comparison alone usually not sensitive enough to pick up differences of potential relevance. Therefore, extrapolation of clinical indications problematic.
Second-generation (next-generation) biological/biologic Biobetter	Biological that has been structurally and/or functionally altered to achieve an improved or different clinical performance.	Usually stand-alone developments with a full development program. Clear (and intended) differences in the structure of the active substance, and most probably different clinical behavior due to, for example, different potency or immunogenicity. From a regulatory perspective, a claim for 'better' would have to be substantiated by data showing a clinically relevant advantage over a first- or previous-generation product.

[a]Comparable terms defined by the same/similar scientific principles include the WHO's 'similar biotherapeutic products' and Health Canada's (Toronto) 'subsequent-entry biologicals'.

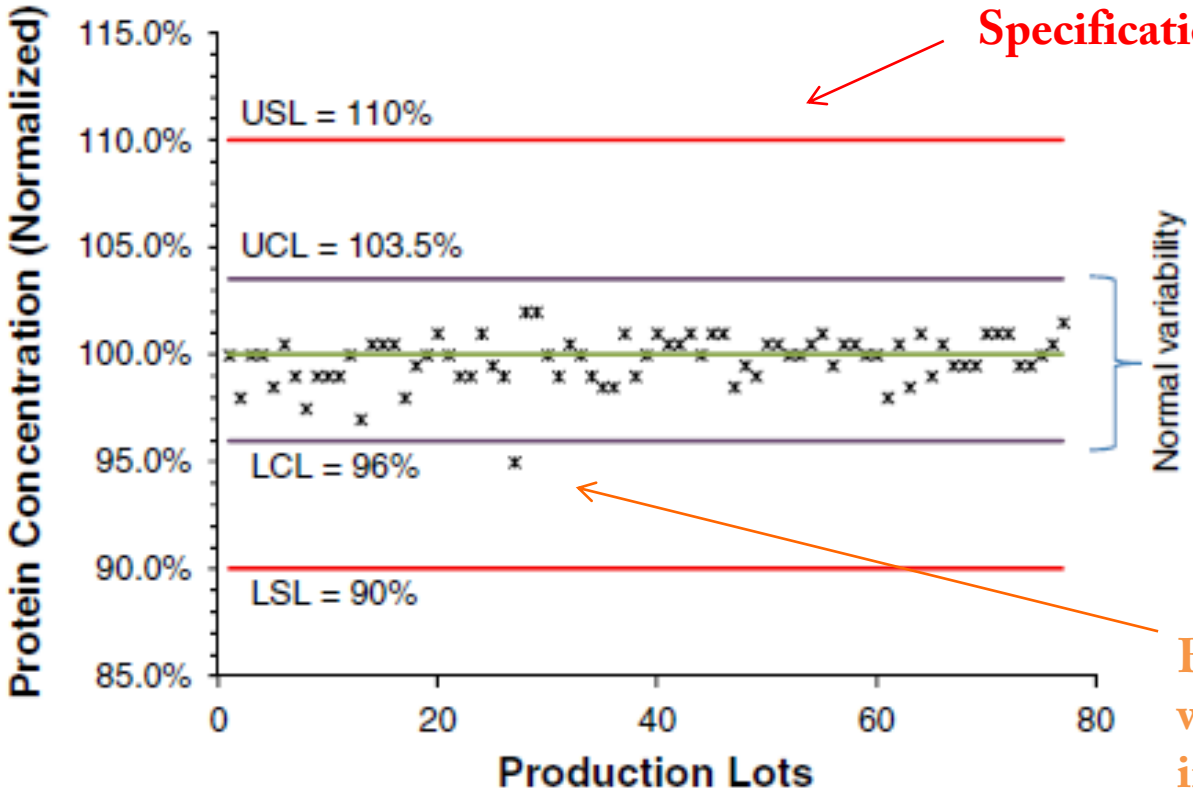
NATURE BIOTECHNOLOGY VOLUME 29 NUMBER 8 AUGUST 2011

It is damn tough to be a Biosimilar…!

„Proven Acceptable Ranges“



„Proven Acceptable Ranges“

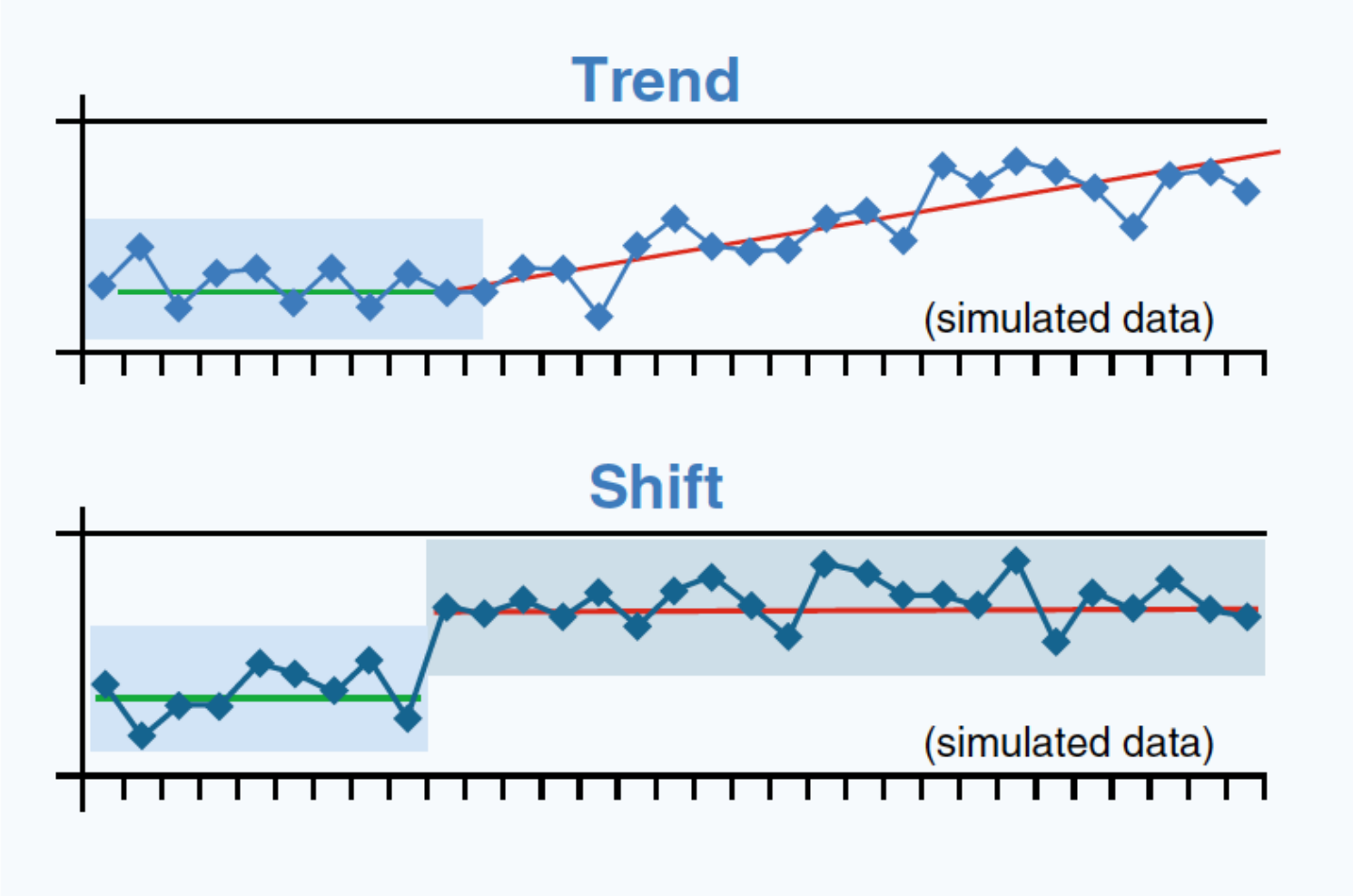


USL/LSL = Upper and Lower Specification Limit

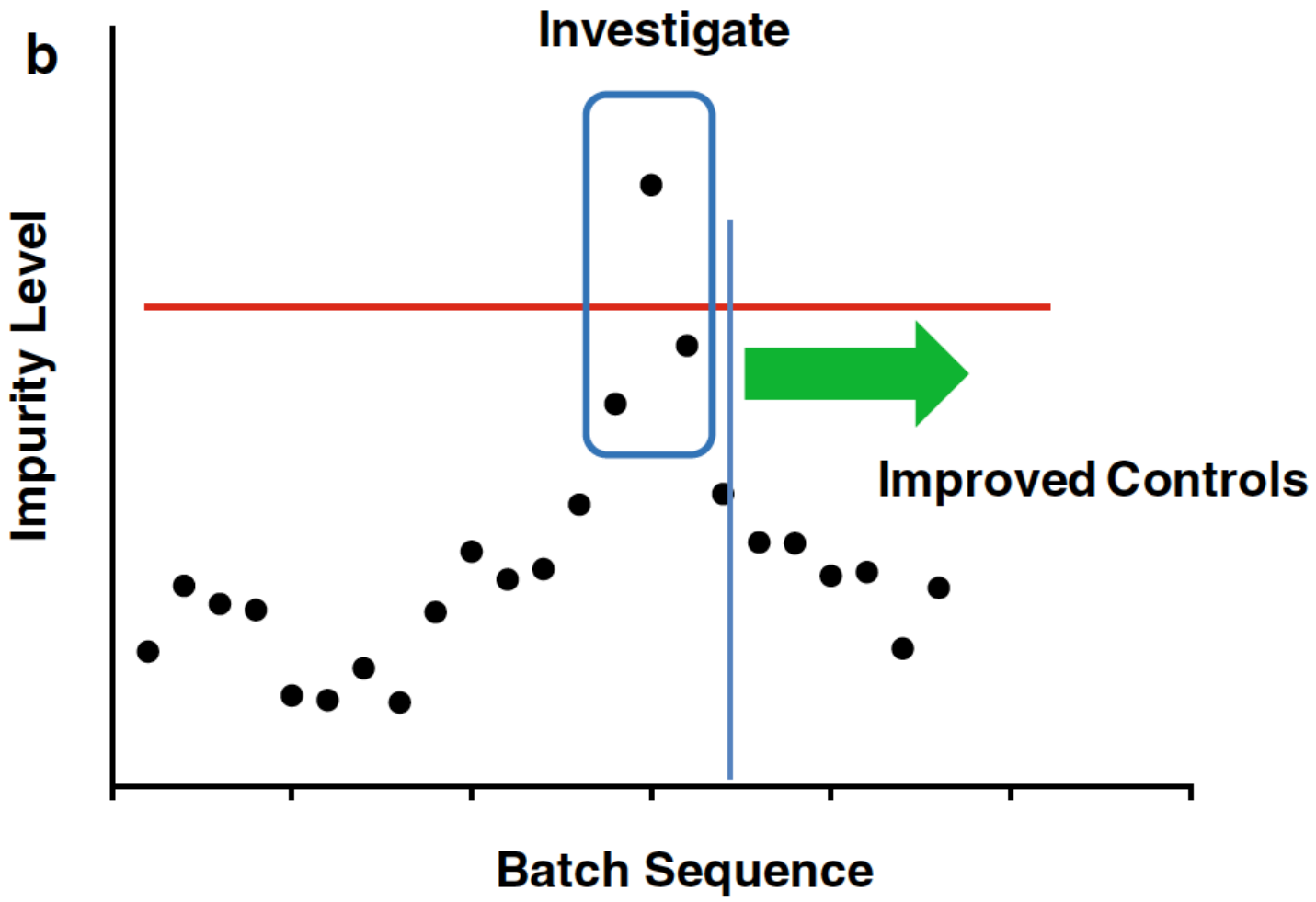
UCL/LCL = Upper and Lower Control Limits on all critical attributes and parameters that are internally trended

Batches out of trend (but within specification) are investigated for potential future drift

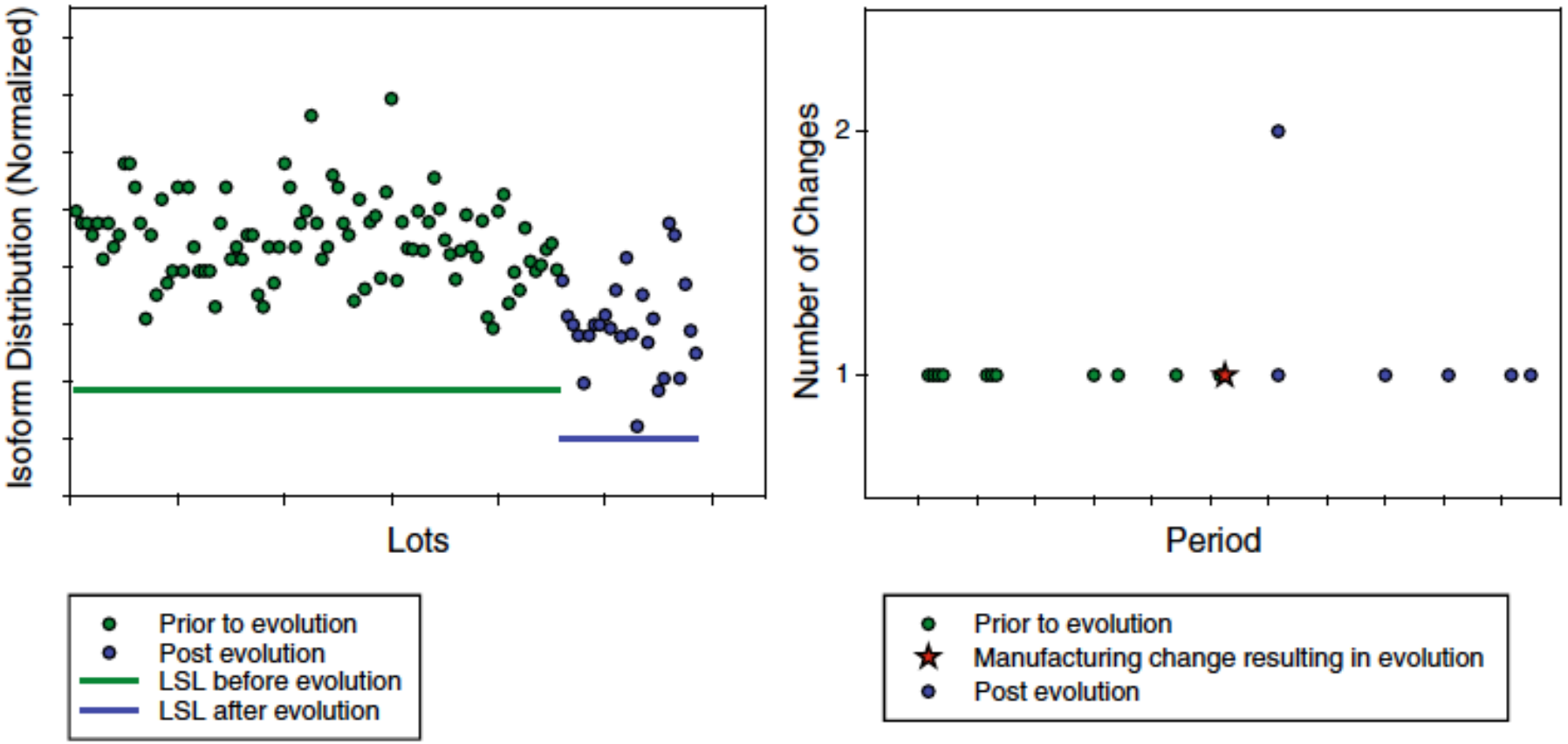
„Proven Acceptable Ranges“: DRIFT



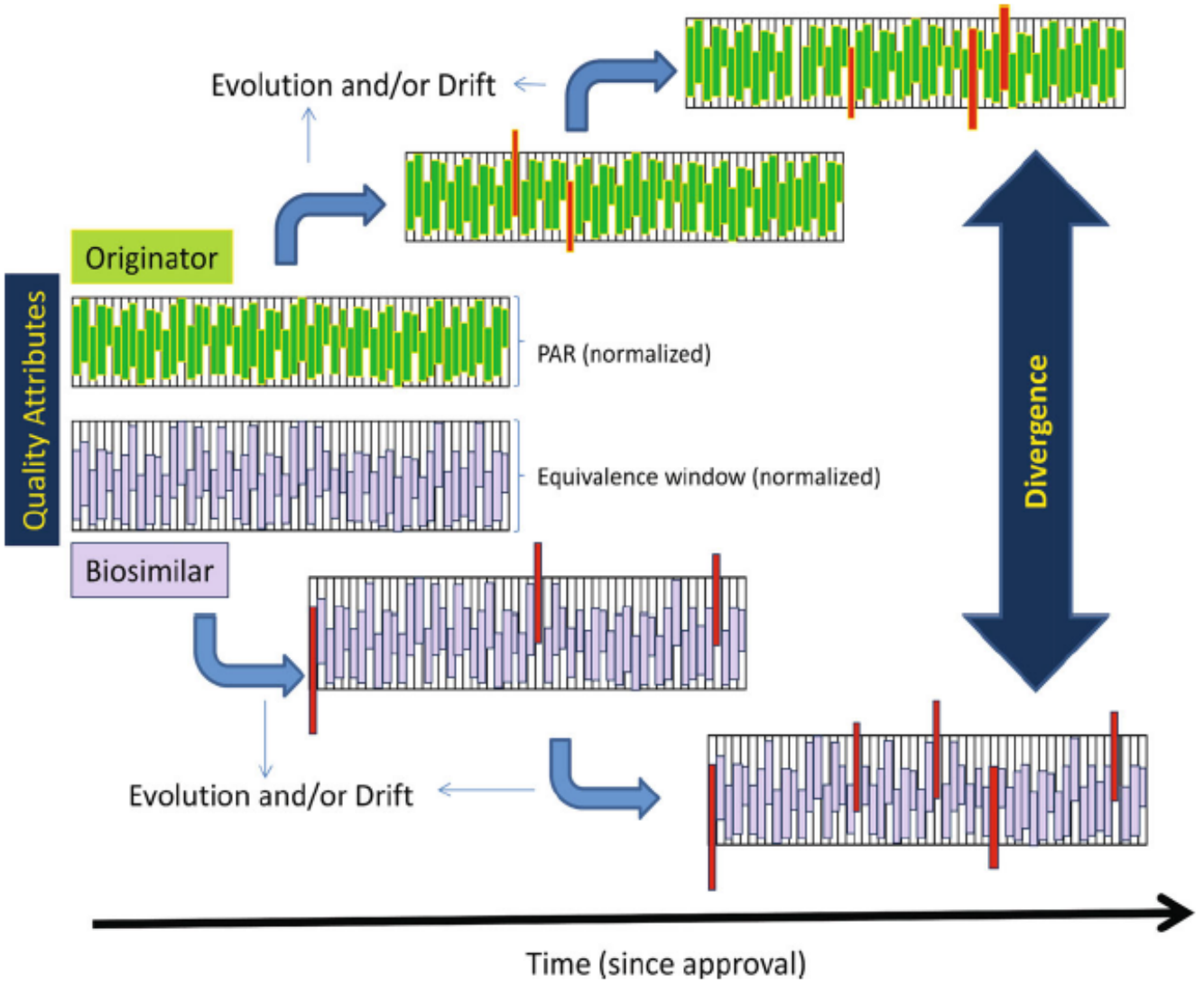
„Proven Acceptable Ranges“: DRIFT



„Batch-to-batch-variation“



„Post-license“ - Evolution



Ramanan & Grampp (2014) BioDrugs.

Market exclusivity and patent safety

Take-home messages

- Biological drugs are either developed as ‘originals’ or ‘biosimilars’
- The production in cell lines is key to success, every cell line/drug has to be developed as an individual entity
- ➔ Biosimilars can only be follow-on products, never be identical copies (≠ generics)
- Immunogenicity is one of the major threats – but this applies to all biolgicals
- „Proven acceptable ranges“ need to be taken into consideration
- Possible changes of biosimilar versus originator need to be monitored

Which of the following microorganisms can be used for the production of biologicals/biosimilars?

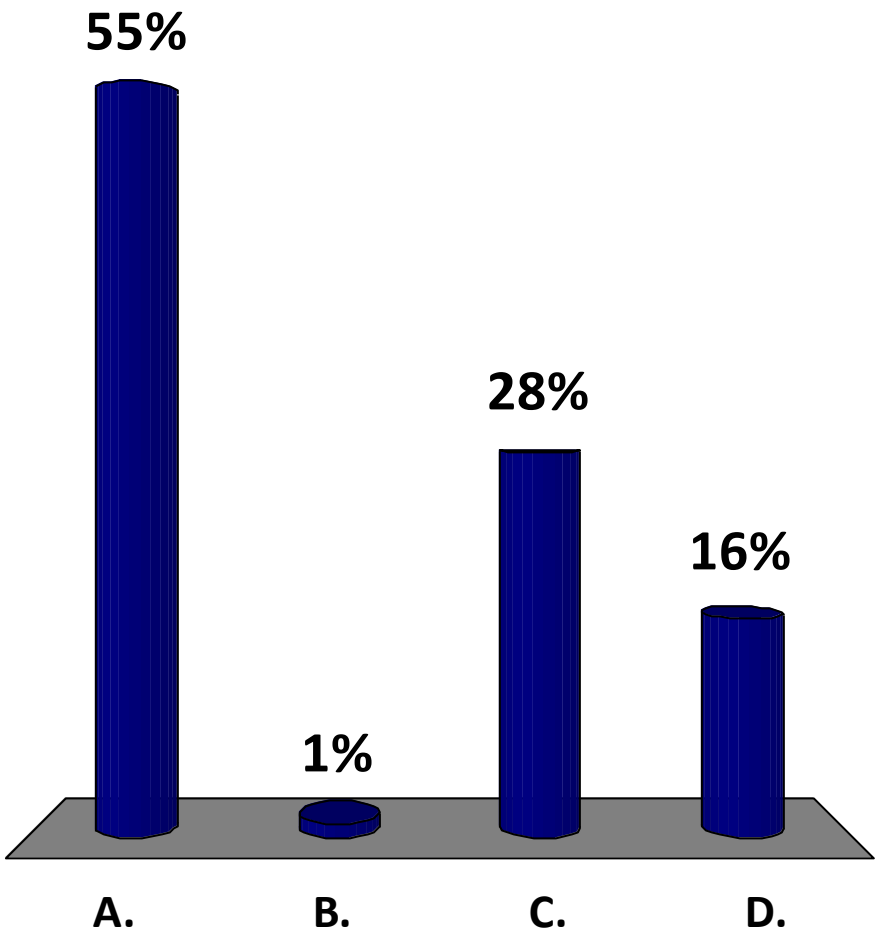
1. Escherichia coli
2. Saccharomyces cerevisiae
3. Chinese hamster ovary cells
4. Spodoptera frugiperda 9 cells

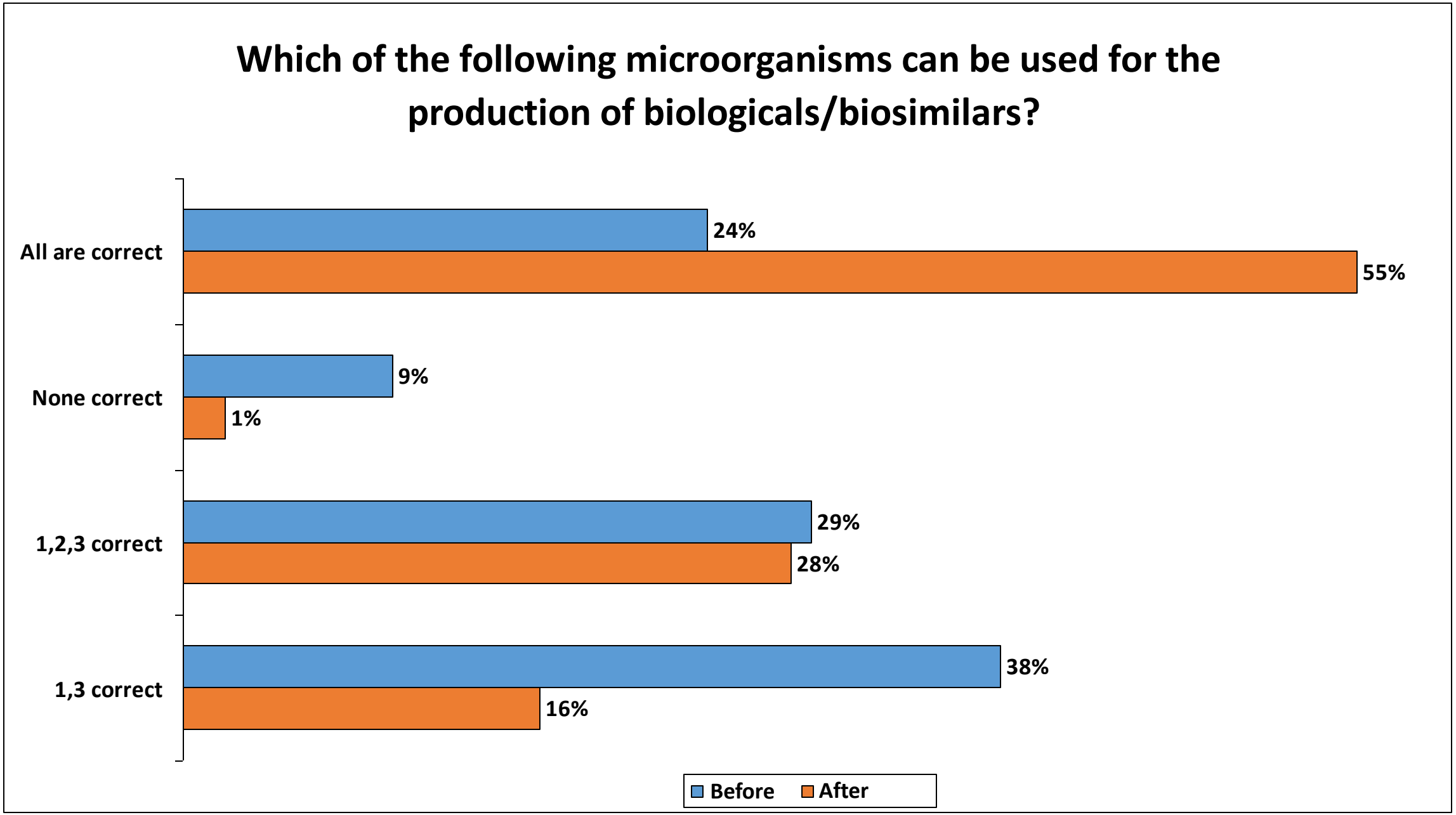
✓ A. All are correct

B. None correct

C. 1,2,3 correct

D. 1,3 correct

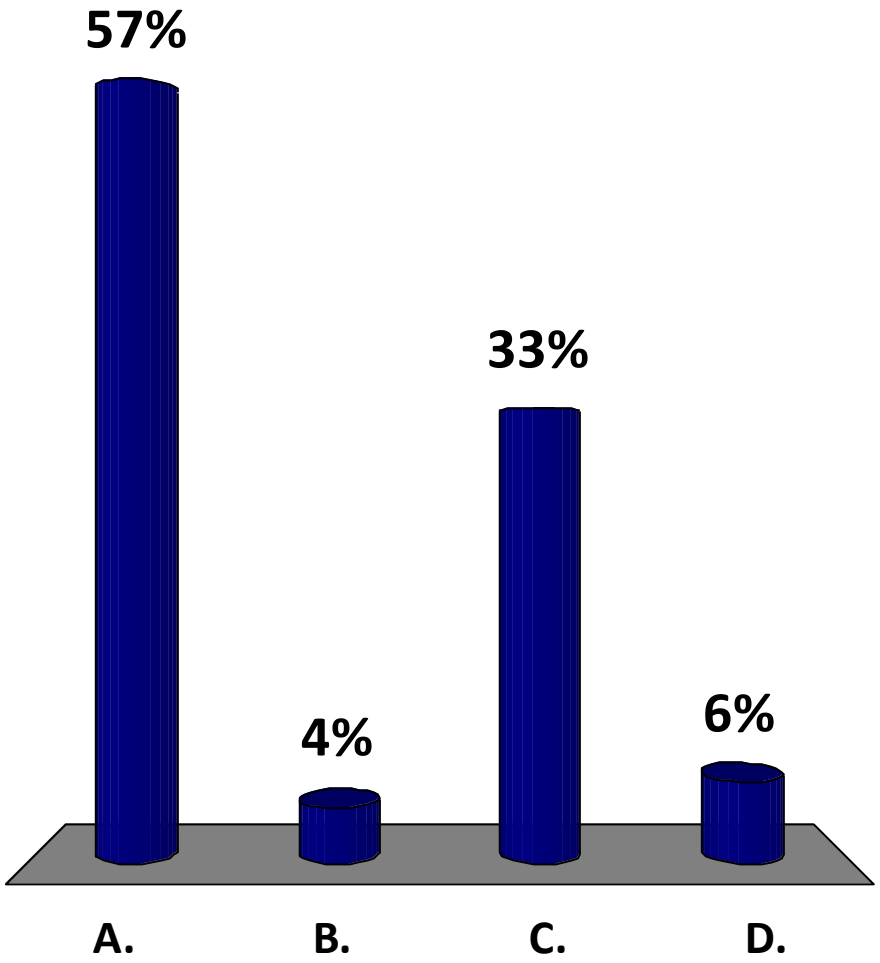


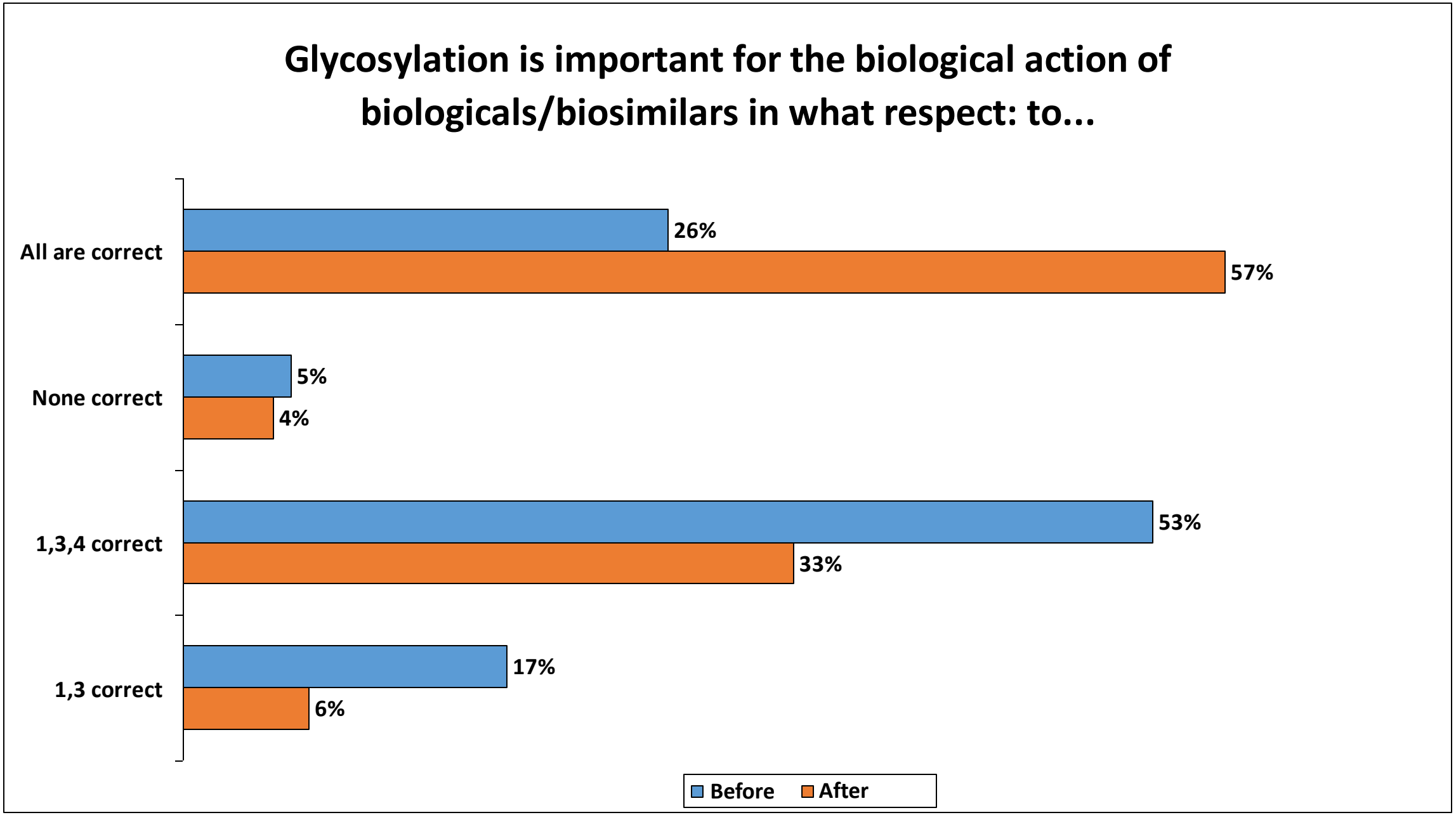

Which of the following microorganisms can be used for the production of biologicals/biosimilars?
All are correct
24%
55%
None correct
9%
1%
1,2,3 correct
29%
28%
1,3 correct
38%
16%
Before
After

Glycosylation is important for the biological action of biologicals/biosimilars in what respect: to...

1. sustain folding
2. increase antigenicity
3. increase stability
4. ensure the folding of the protein

A. All are correct
B. None correct
✓ C. 1,3,4 correct
D. 1,3 correct




Glycosylation is important for the biological action of biologicals/biosimilars in what respect: to...
All are correct
26%
57%
None correct
5%
4%
1,3,4 correct
53%
33%
1,3 correct
17%
6%
Before
After

The quality attributes of biological/biosimilar medicines include:

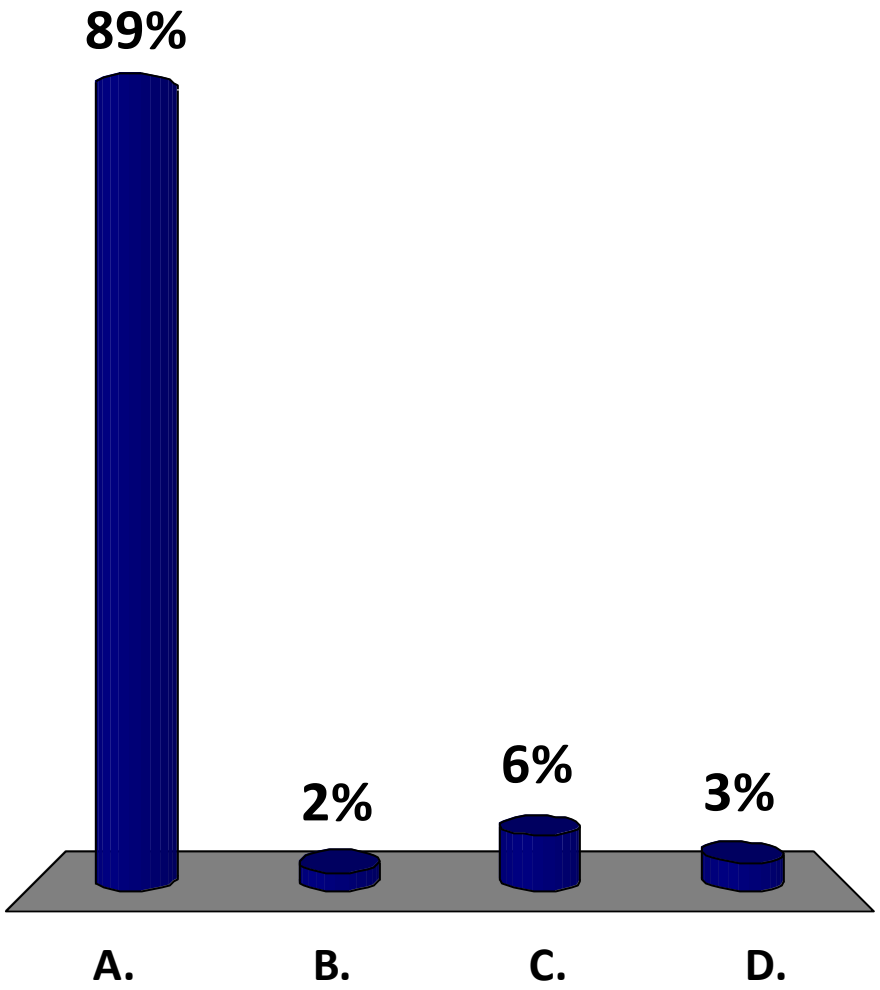
1. primary structure
2. particles and aggregates
3. imurities
4. stability

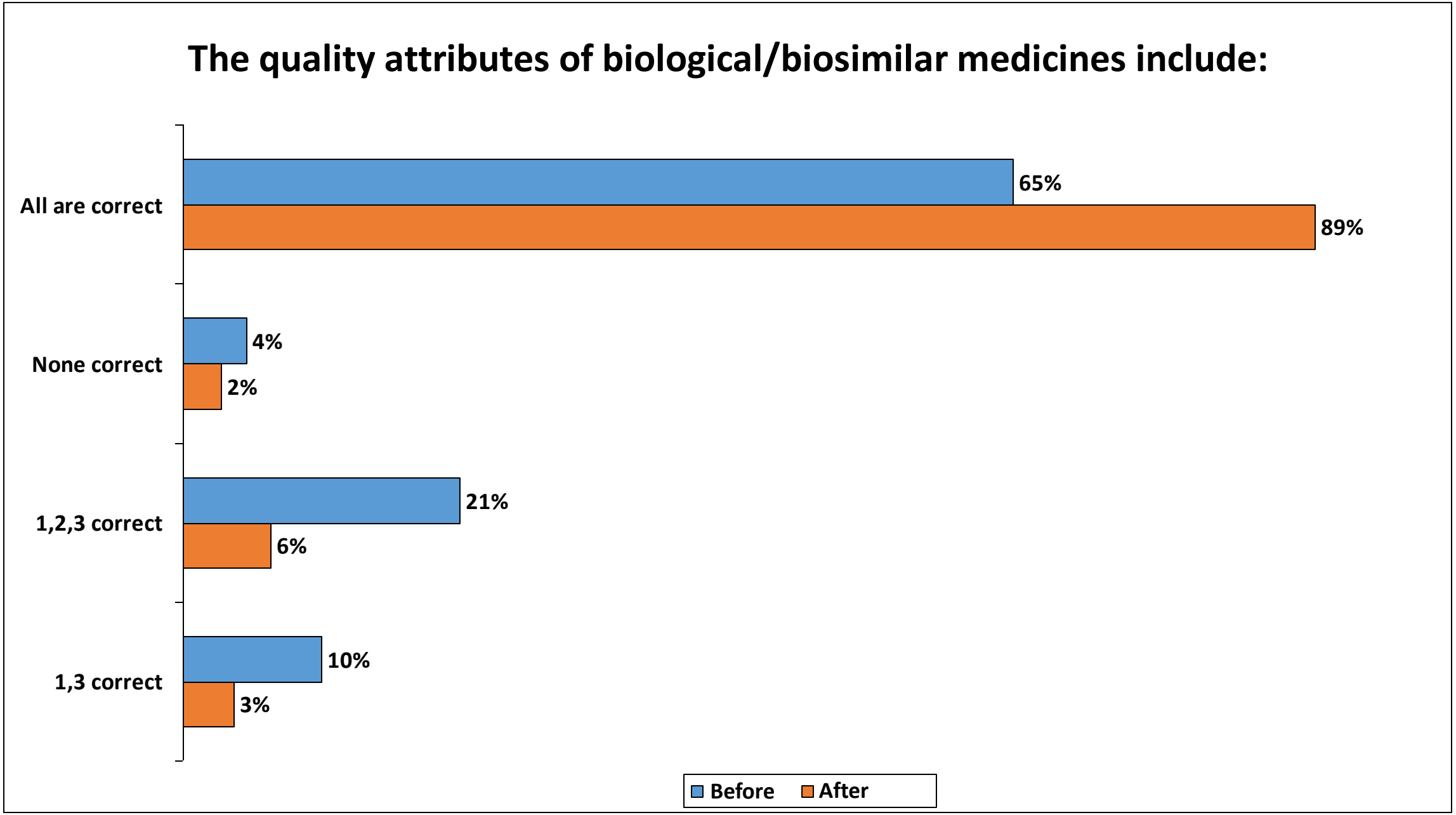
✓ A. All are correct

B. None correct

C. 1,2,3 correct

D. 1,3 correct




The quality attributes of biological/biosimilar medicines include:
All are correct
65%
89%
None correct
4%
2%
1,2,3 correct
21%
6%
1,3 correct
10%
3%
Before
After