ebe european biopharmaceutical enterprises

EUROPABIO The European Association for Bioindustries

**EMEA Workshop on Biosimilar Monoclonal Antibodies, July 2, 2009**

# Session 1 CMC
# Innovator Industry Presentation

**Prof. Georg-B. Kresse**

# The Innovators‘ View – CMC Summary

- **Available guidance for quality characterization is applicable for biosimilar mAbs**
- **The mode of action of mAbs is complex and may involve contributions from multiple mechanisms**
- **Differences in antibody variants are only acceptable if justified by clinical data**
- **Glycosylation can be critical for the biological function of mAbs**
- **Quality data cannot substitute for gaps in knowledge in functional assays**
- **Role of ICH Q8 and Q9: a „design space“ concept is not transferable from the reference product to a biosimilar to ensure similarity**

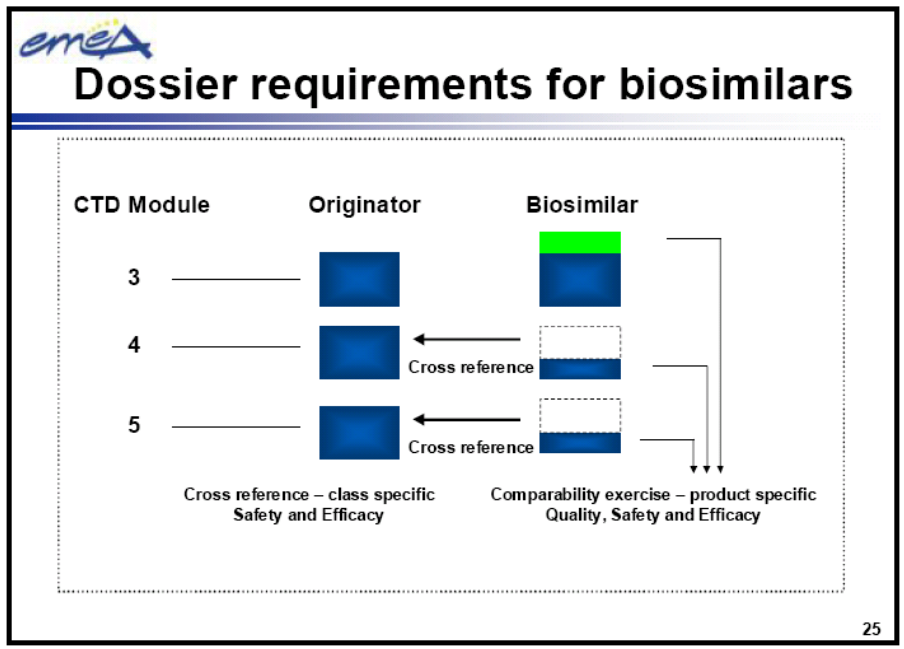
# The EU Biosimilars Existing Regulatory Framework

• It is **impossible** to characterize the quality attributes of proteins completely by physicochemical analysis, and to fully **predict the impact of structural differences** (induced by the producing cell itself as well as by the manufacturing process) on clinical efficacy and safety.

• For independently manufactured protein products, **„identical copies“** are impossible but at best „similar“ products. Biosimilars are manufactured and controlled according to their own development. Similarity has to be shown in terms of **quality, efficacy, and safety** in head-to-head comparative studies.

• Analytical **differences** between biosimilars and their reference product are **expected.** The extent of observed differences will be decisive for definition of the non-clinical and clinical program.

• The „**biosimilarity**“ scenario differs from the „**comparability after manufacturing changes**“ scenario regulated by ICH Q5E.

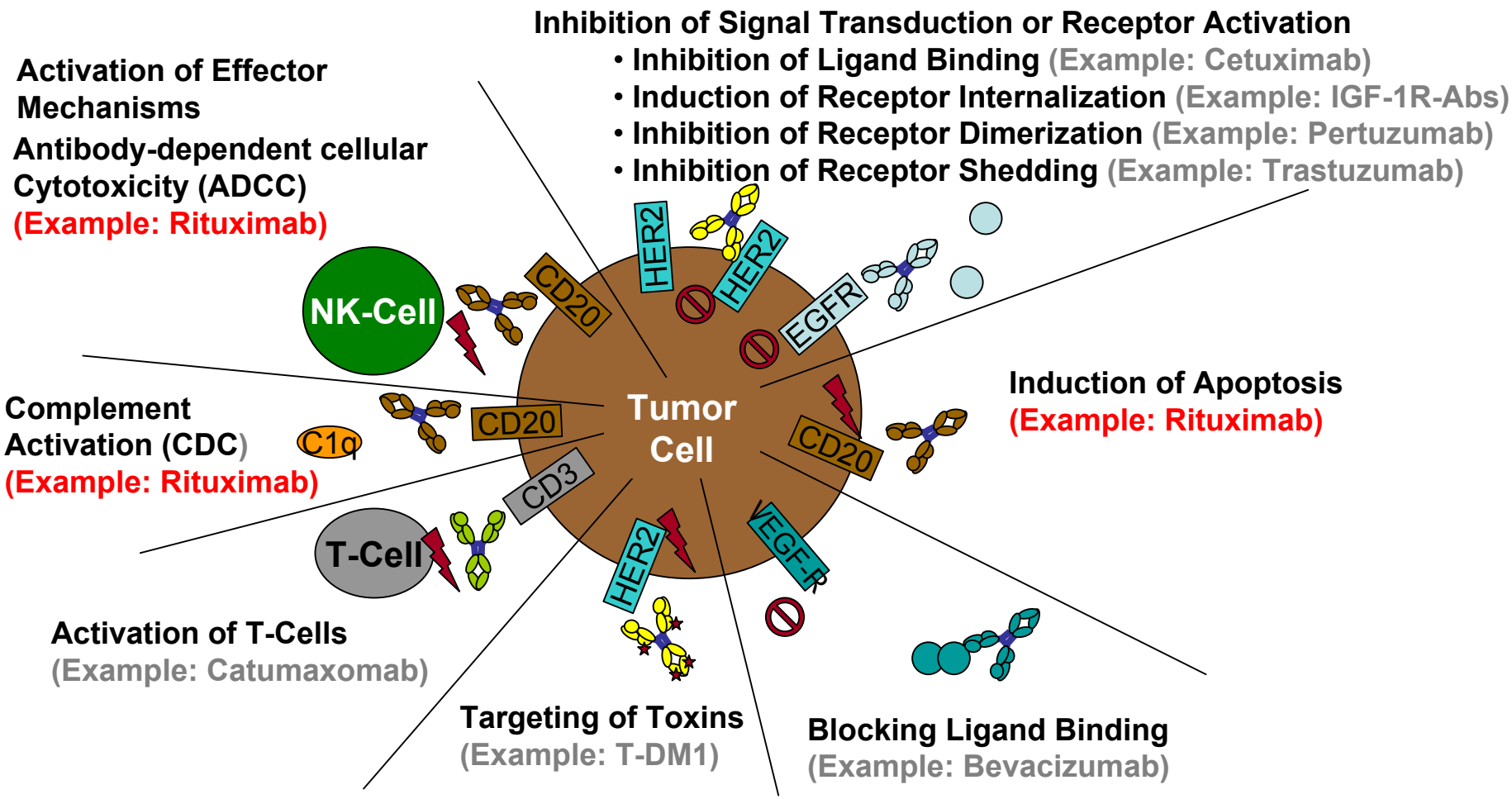



Source: P. Richardson and P. Celis (2007), 1st Drug Evaluation Forum, Pharm. Soc. Japan

# Available Guidance for Quality Characterization is applicable for biosimilar mAbs

- **The same principles should apply** for quality characterization of biosimilar mAbs as for other biosimilars.
- Available guidelines on **Quality Characterization of mAbs** (CHMP/BWP/157653/2007) and on **Quality Issues of Biosimilar Products** (CHMP/BWP/49348/2005) are applicable for biosimilar mAbs. There is no need for additional quality guidance.
- State-of-the-art analytical methods will have to be used. However, with more sensitive analytics, **more differences will be detected**. On the other hand, even extended analytical characterization is limited – **„you will only see what you are looking for".**
- Both **physicochemical and biological assays** will be necessary in all cases.
- The quality studies have to take into account **multifunctionality** of mAb molecules and should include in-vitro **potency assays** (if predictive assays are available) as well as assays for **Fab and Fc mediated functions** as needed based on an understanding of the mechanism of action of the particular mAb. Assumptions based e.g. on the IgG sub-class are not sufficient.

# The Mode of Action of mAbs is complex and may involve Contributions from multiple Mechanisms



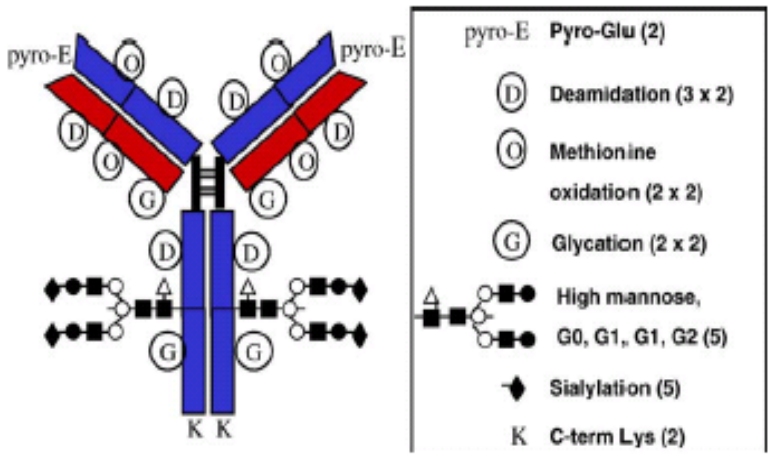

**The *in-vivo* net contribution of different modes of action described for one mAb is often incompletely understood and may also be different in different indications.**



Modified from: Hasmann, M. et al. (2009) ChiuZ, submitted

# Differences in Antibody Variants are only acceptable if justified by clinical Data

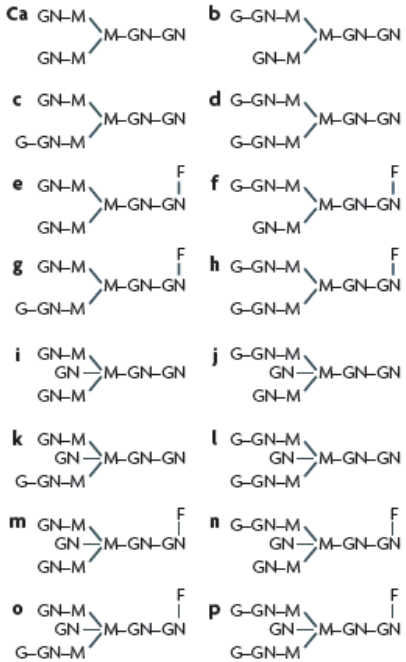
- Biosimilars **must have the same amino acid sequence** as the reference product.
- The **relevance** of major variants (e.g., basic/acidic variants, presence or absence of C-terminal Lys residues) **on clinical efficacy and/or safety** has to be established and reflected in the control strategy of the product.
- mAbs (reference and biosimilar) will always be **micro-heterogeous mixtures** of a large number of post-translationally modified molecular species.
- The exact composition of this mixture **cannot be reproduced** if a different manufacturing process is used, therefore **comparative non-clinical and clinical data** will always be necessary for biosimilar mAbs.




Not all possible variants are described. For example, there are fucosylation variants in glycosylation that were not counted. If one assumes these variants are independent and considers combinations, each half-antibody has 2x6x4x4x5x5x2=9600 possible states. If one assumes both halves of the antibody are independent, there are $(9600)^2 \approx$ **$10^8$ possible states**.

Source: Kozlowski, S. & Swann, P. (2006) Adv. Drug Delivery Revs 58, 707-722

# Glycosylation can be critical for the biological Function of mAbs




Source: Jefferis, R. (2009) Nature Revs. Drug Disc. **8**, 226-234

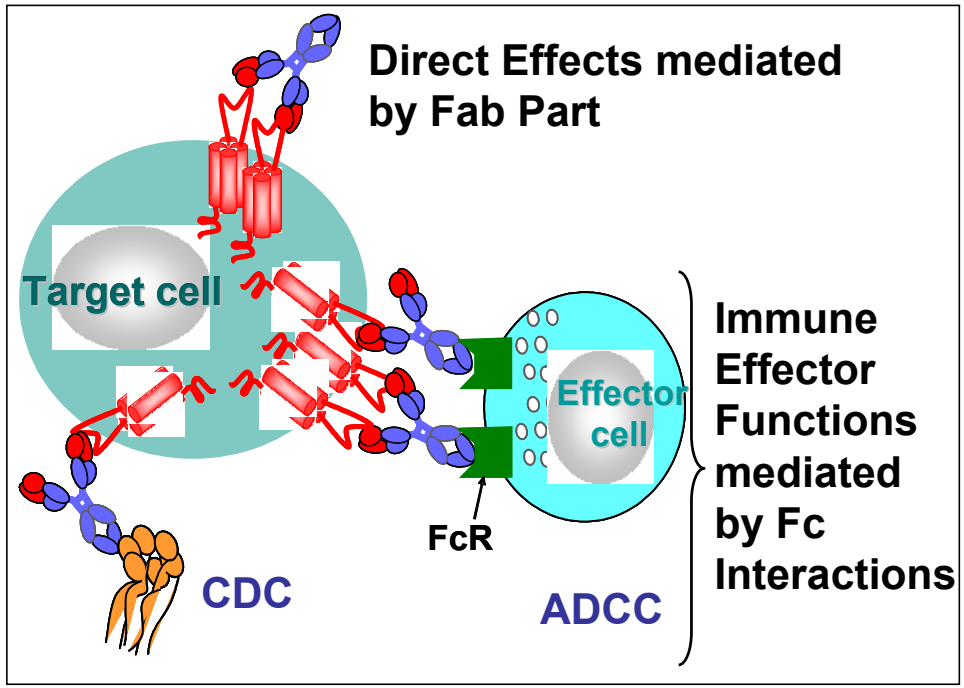
- IgGs may contain up to **~500 different glycoforms** due to **Fc glycosylation**. Differences may influence solubility, stability, clearance, immunogenicity, and immune effector functions.
- Even **small differences in glycosylation** may have significant effects (e.g., impact of absence of one fucose residue on ADCC - however, not only fucosylation is important).
- Up to 30% of human IgGs contain N-linked **oligosaccharides in the Fab region** whose functional significance is not fully evaluated (e.g., impact of Fab galactosylation on hypersensitivity reaction).

- The **pattern of glycosylation will vary** between products because it depends on the manufacturing process.
- In line with the mAb guidance, the relevance and acceptability of glycosylation differences should depend on **criticality of this attribute**, i.e. the proven or disproven **impact of glycosylation differences on clinical properties** which may be different for mAbs using different modes of action.

# Need for functional Assays - Quality Data cannot substitute for Gaps in Knowledge

• The **broad experience** existing with therapeutic mAbs shows that it may be **difficult to understand the critical quality attributes**, and to predict the impact of differences to **clinical efficacy and safety** (e.g., Raptiva example).

• Only those differences **known/proven to have no impact** on clinical efficacy and/or safety should be acceptable without additional justification.



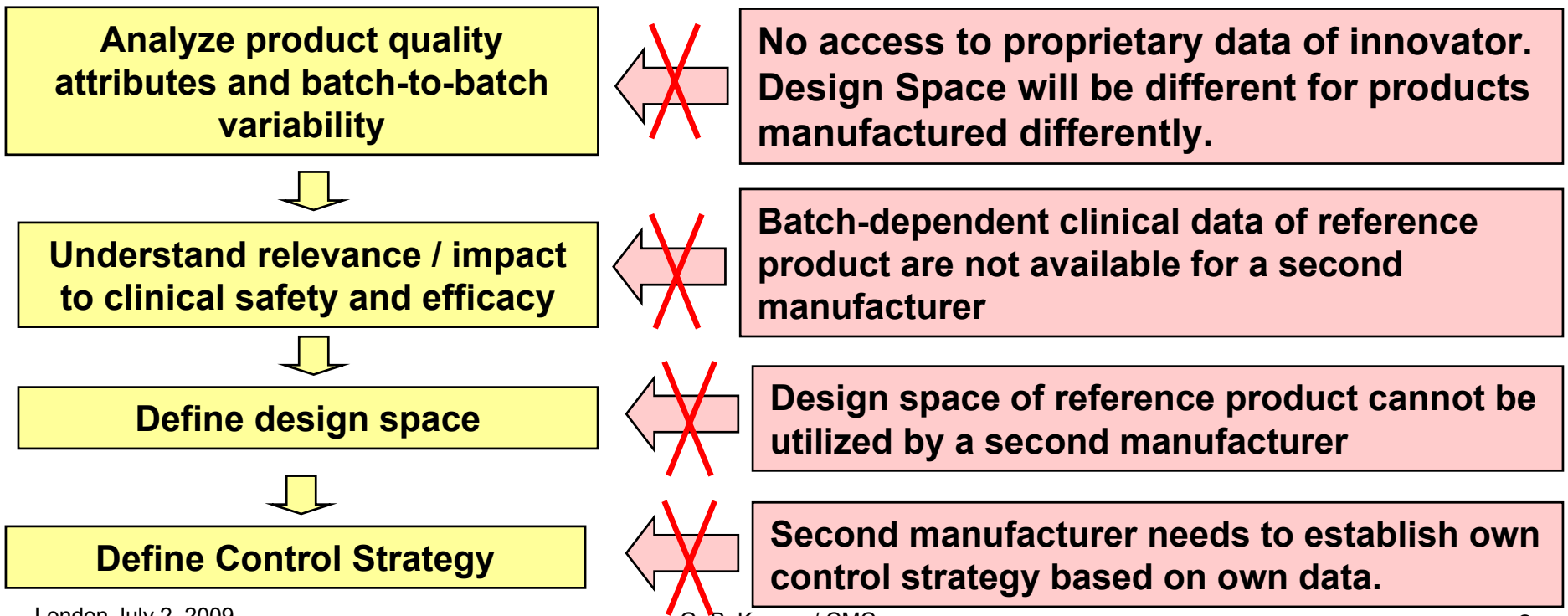

• Gaps of functional knowledge will lead to the requirement for **additional non-clinical and clinical data**, based on knowledge of the mode of action of the particular mAb under study.

• Because of the linkage of quality, non-clinical and clinical aspects, a **„holistic approach“** is needed for the evaluation of mAb-based drugs to connect analytical data with clinical safety and efficacy.

# Role of ICH Q8 and Q9: A „Design Space“ Concept is not applicable to ensure Similarity

• ICH Q8 **Pharmaceutical Development** and ICH Q9 **Quality Risk Management** are applicable for biosimilars manufacturers for their own developments in the same way as for the originators.

• A „**design space**“ depends on a particular manufacturing process connected to clinical results and cannot be „borrowed“ to demonstrate **similarity of a biosimilar product** to a reference product made by a different process.

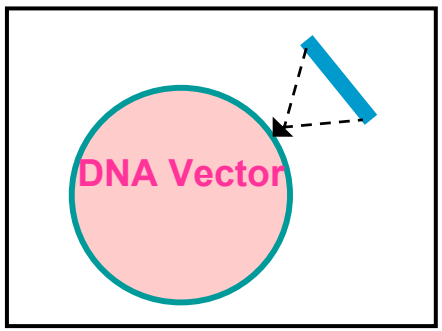


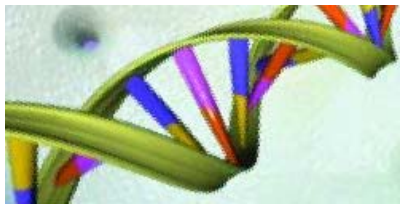
# CMC Conclusion

- Available **quality guidance** is applicable for biosimilar mAbs.
- It is **impossible to characterize the quality attributes of mAbs completely** by physico-chemical analysis alone and to fully **predict the impact of differences** on clinical efficacy and safety. Analytical differences (including glycosylation if appropriate) have to be justified by clinical data.
- **Multifunctionality** of mAb molecules means that characterization should include both **Fab and Fc mediated functions** unless justified.
- The **mode of Action** of mAbs is often complex involving contributions from multiple mechanisms (these may be different in different indications).
- mAbs will always be **micro-heterogeneous mixtures** of a large number of molecular species. The **relevance** of major variants **on clinical efficacy and/or safety** has to be established and reflected in the control strategy of the product.
- **ICH Q8 and Q9** are applicable for biosimilars manufacturers for their own developments as for originators. However, a „design space“ concept will not help to establish similarity.

# Back-up Slides

# Manufacturing of recombinant Proteins is complex



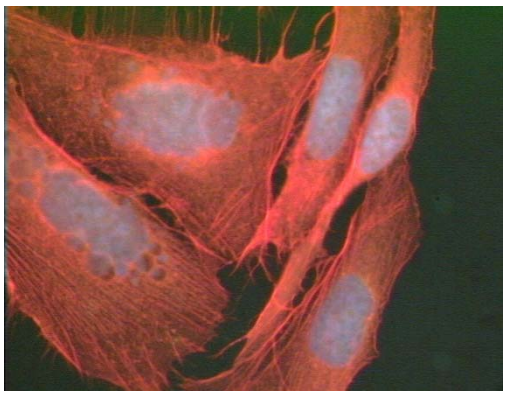



Cloning into
DNA Vector

Downstreaming

Transfer into Host Cell
Expression
Cell Development

Large-Scale Fermentation

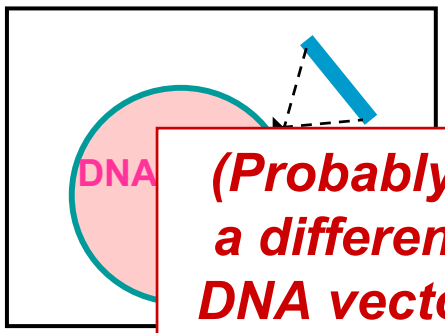


e.g., bacterial or mammalian cell

Formulation

# Manufacturing of recombinant Proteins is complex

A second manufacturer uses...




*(Probably) a different DNA vector*

Cloning into DNA Vector

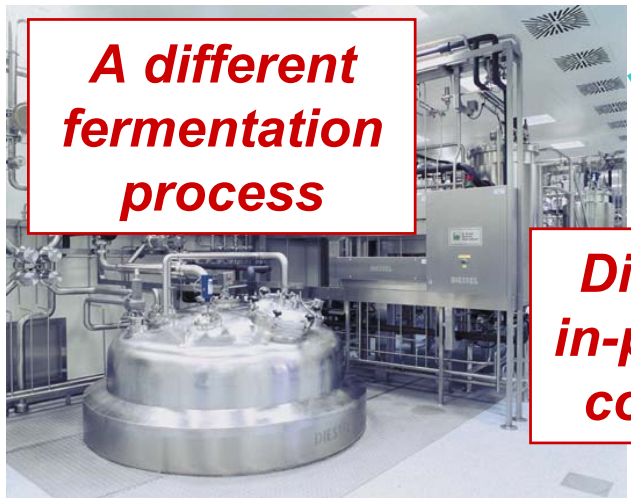
*The same amino acid sequence*
*(maybe the same genetic sequence)*

*A different downstreaming protocol*

Downstreaming

Transfer into Host Cell
Expression
Cell Development

*A different fermentation process*



Large-Scale Fermentation

*Different in-process controls*

*A different recombinant cell expression system*

e.g.,

*Maybe a different formulation*

Formulation

**Different Process → different product**

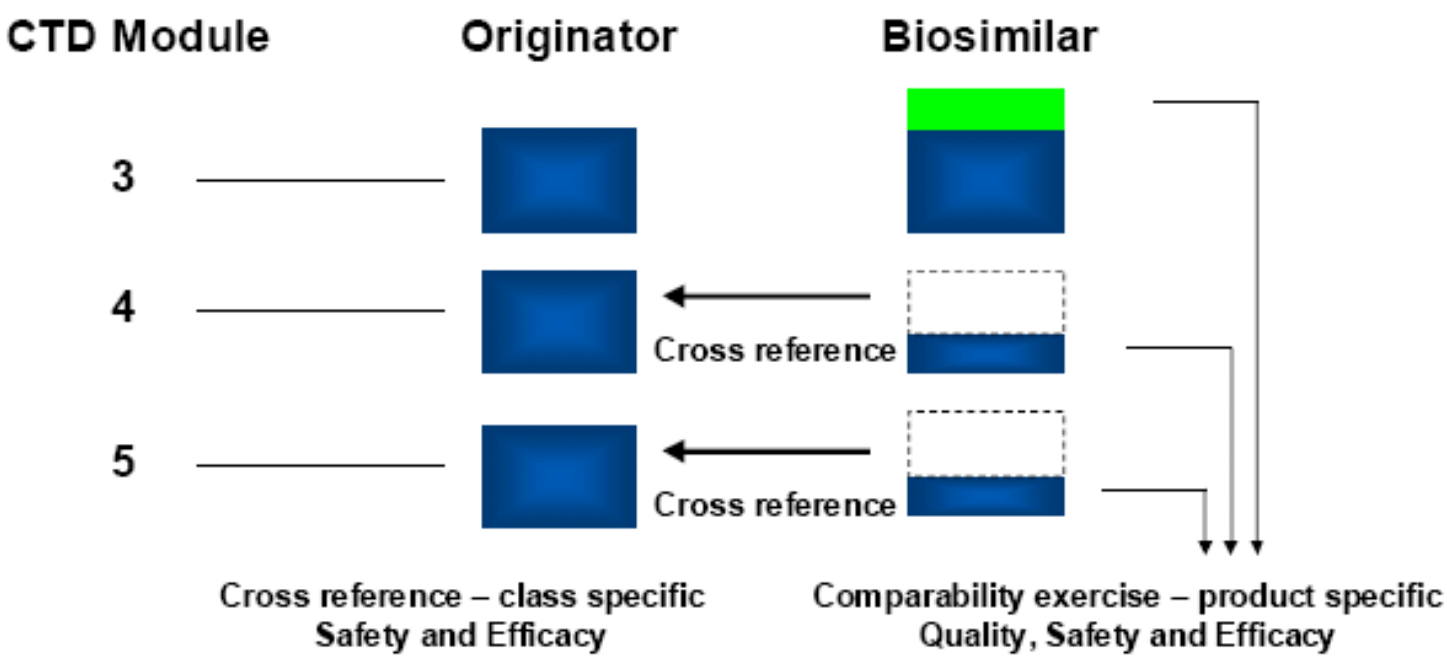
# Are mAbs „well-characterized Biologicals“ ?

• The term **„well-characterized biological“** should not be used because is not defined in any EMEA guideline.

• It was originally introduced by FDA in 1995 but soon abandoned:

> In the Federal Register of December 8, 1995 (60 FR 63048), the agency first published an **interim definition of a well-characterized therapeutic recombinant DNA-derived and monoclonal antibody biotechnology Product** … After considering the public comments received on the interim definition … and the many requests the agency has received for further clarification of the term ‘‘well-characterized,’’ FDA has determined that **it may not be possible to achieve a sufficiently clear and specific understanding of this term** to adequately apprise potential applicants of the applicability of the new procedures. Accordingly, FDA is specifying, in lieu of the term ‘‘well characterized biotechnology product,’’ the categories of products to which this final rule will be applicable.” (Federal Register / Vol. 61, No. 94 / Tuesday, May 14, 1996)

# Dossier requirements for biosimilars

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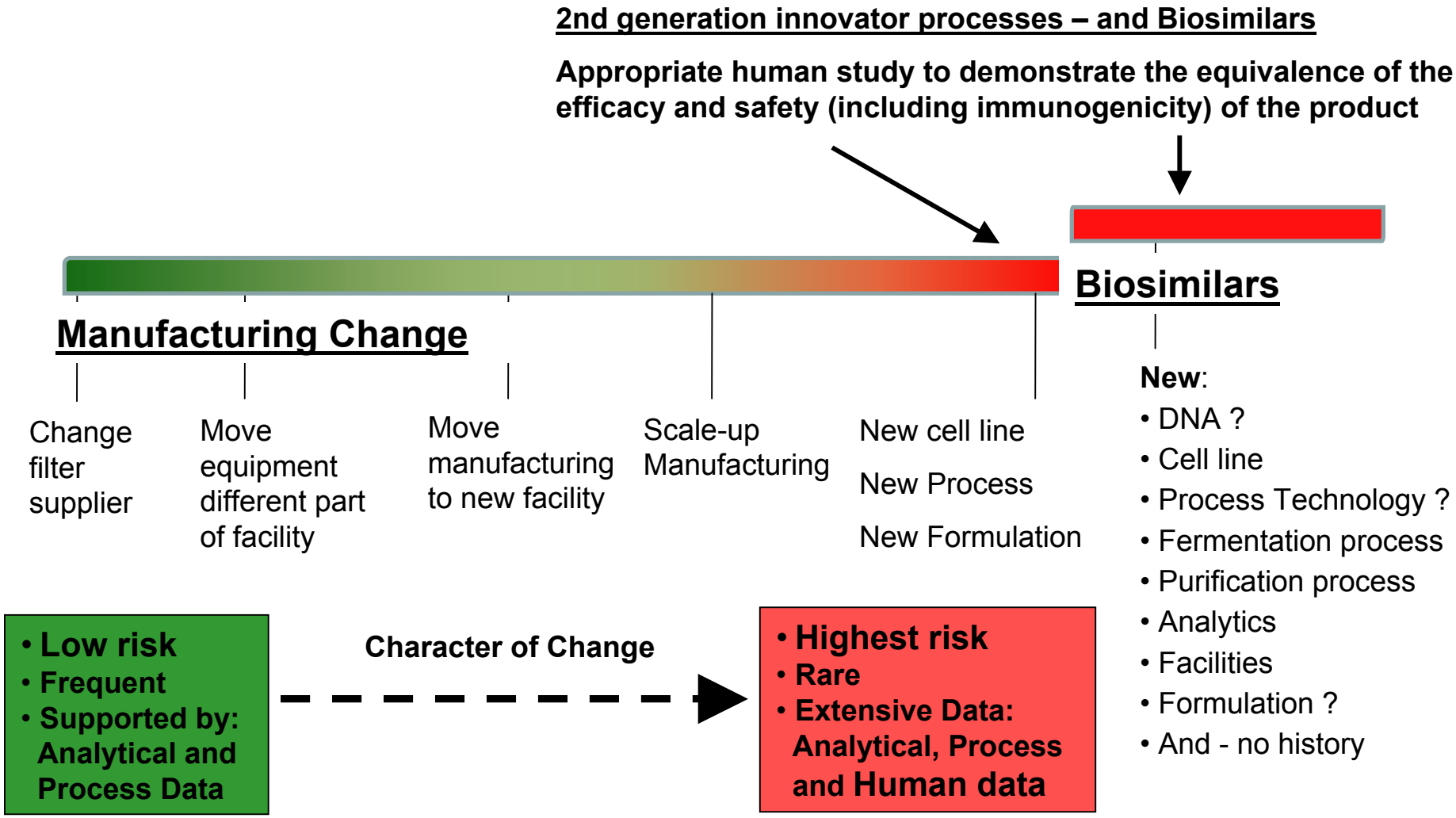
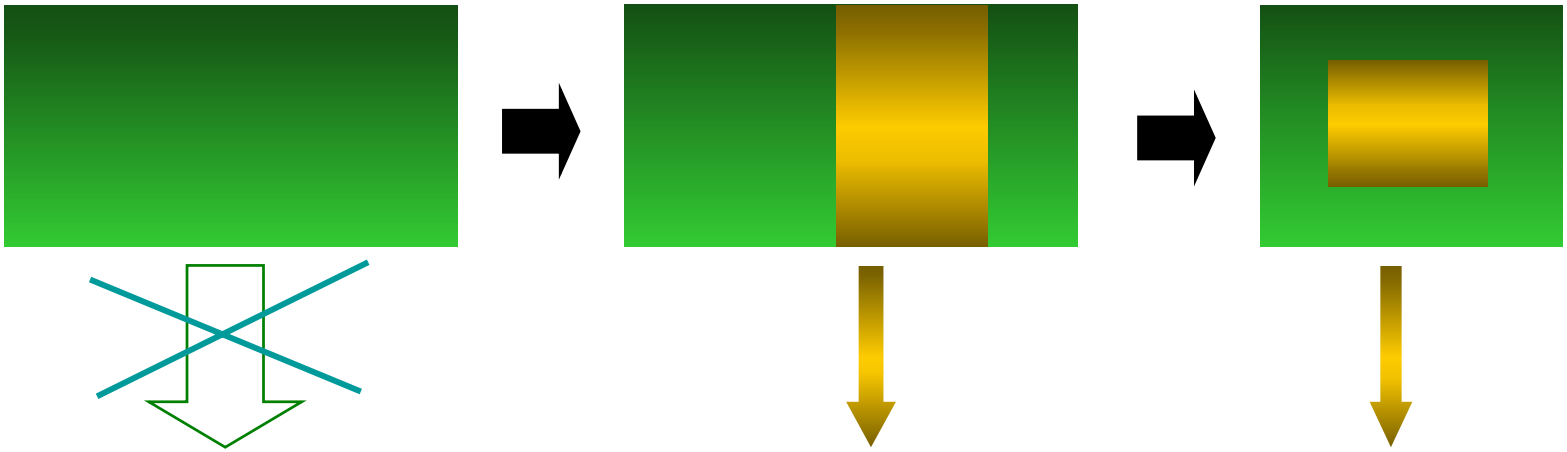
Source: P. Richardson and P. Celis (2007), 1st Drug Evaluation Forum, Pharm. Soc. Japan

# Standard for documenting Innovator Process Changes is well established – Standard for Biosimilars should be no less




Source: modified from Ken Seamon, Cambridge University

# Second Manufacturers have no Access to the Innovator‘s Database




**Second Manufacturer**

**No access to historical database**

**No definition of “design space” and assessment of similarity possible**

**Limited access to historical database**

**Case-by-case: clinical development can be shortened**

**Comparison at the level of final product only (limited to drug product level)**

Trade secret of innovator

Accessible for independent second manufacturer

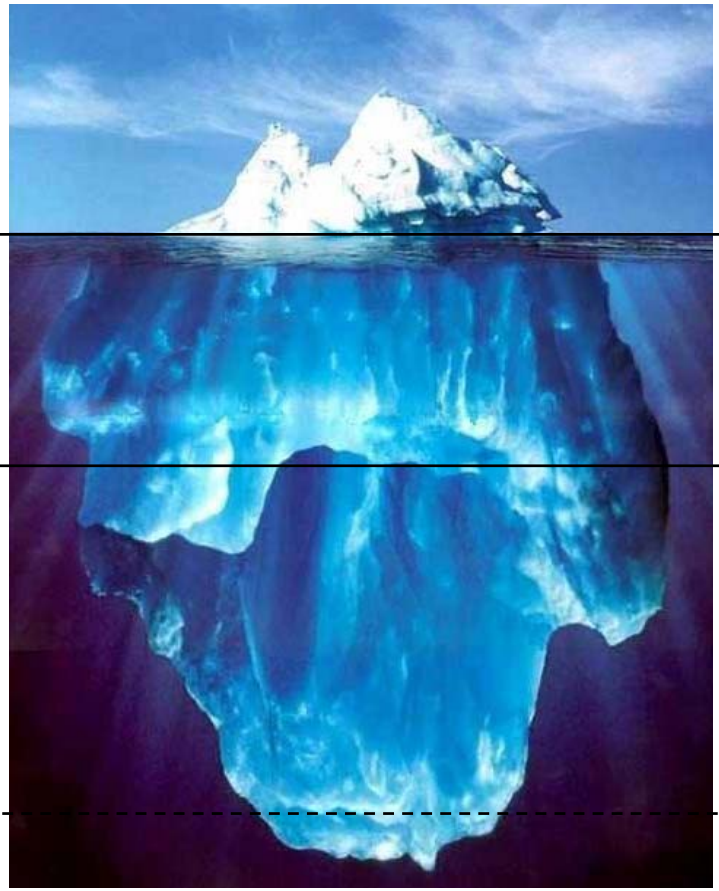
# How much of the Iceberg is visible?

**Release Tests (Specifications)**

**Extended Characterization**
**(Process & Product)**

**Process Control**
- Procedures
- Materials
- In-process testing
- Monitoring
- Validation

**Unknown**
**Learned over time – update control strategy**



*Consistency*

*Similarity*

*Comparability*

**End-Product only**

**In-Process Controls**

Modified from: Koszlowski, S. & Swann, P. (2006) Adv. Drug Delivery Revs. **58**, 707-722

# Is physicochemical Characterization sensitive enough to detect Differences between two more complex Molecules like mAbs ?

- **Sensitivity is not the issue** – the analytical toolbox becomes more and more comprehensive, and state-of-the-art analytical methods have to be used by biosimilars developers as well as by innovators.
- With more sensivity analytics, **more differences will be detected** - not „more identity“.

**„The greater the resolution of the analytical methods applied to a product, the more heterogeneity will be apparent. The critical issue is which variants matter and at what levels they matter“**

Kozlowski, S. & Swann, P. (2006) Adv. Drug Delivery Revs. 58, 707-722

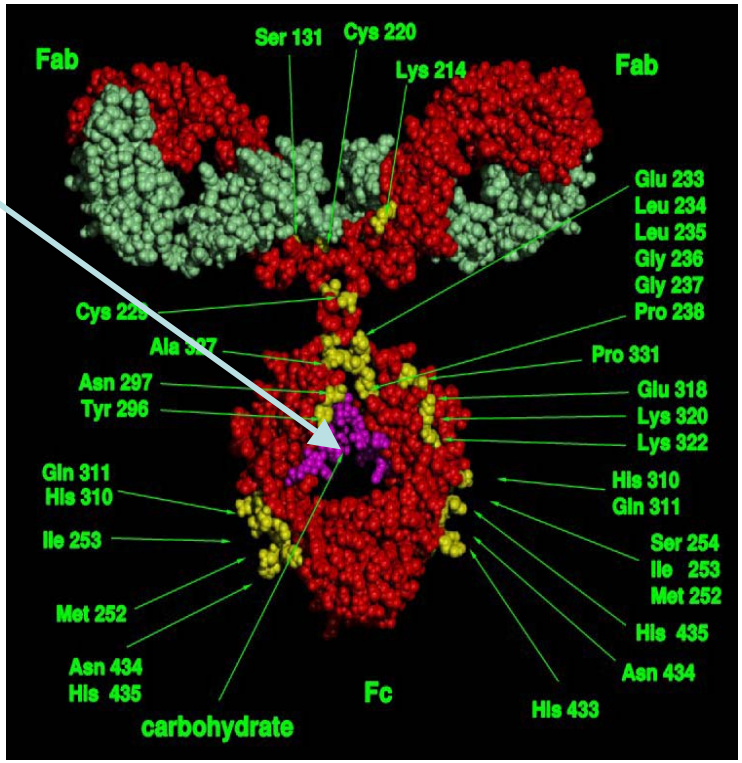
- According to the biosimilars quality guideline, **differences are expected** anyway – but the extent of observed differences decisive for definition of the non-clinical and clinical program.

It is **not expected that the quality attributes in the similar biological and reference medicinal products will be identical**. For example, minor structural differences in the active substance, such as variability in post-translational modifications may be acceptable, however, must be justified. … Therefore, differences … may have **consequences with regard to the amount of non-clinical and clinical data** which may be required in order to make satisfactory justification of the safety and efficacy of the similar biological medicinal product.

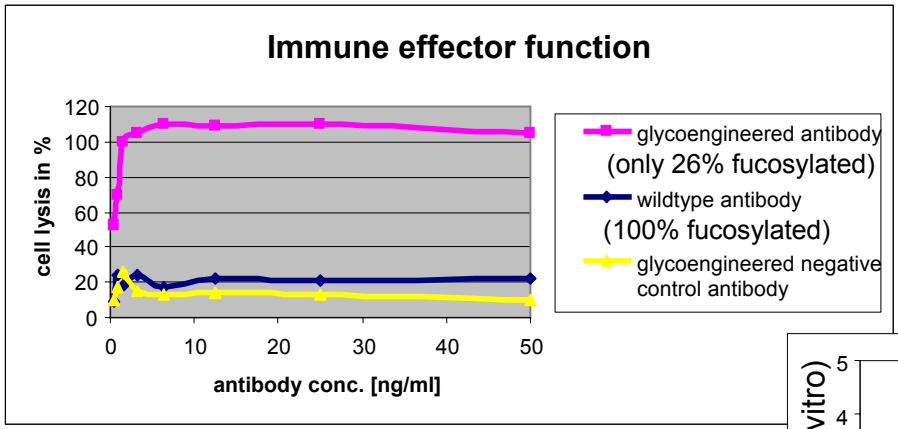
EMEA/CHMP/49348/05

# Even small Glycosylation Differences may have significant Effects on Immune Effector Functions

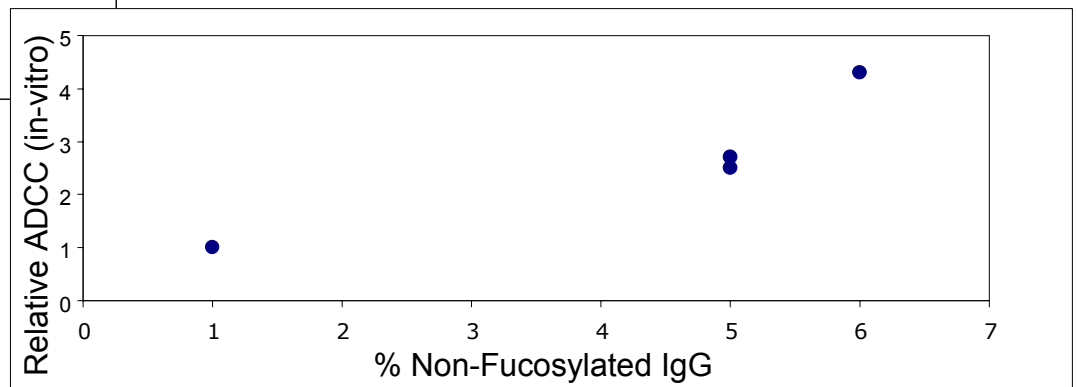
- Both **amino acid sequence and glycosylation pattern** of $C_H2$ influence FcR binding and ADCC activity.
- The presence or absence of **one fucose residue** can affect the biological activity (killing of target cells via ADCC).
- Even **very small differences in fucosylation** may have significant effects on *in vitro* ADCC.




Clark, M., http://wwwimmuno.path.cam.ac.uk/~mrc7/

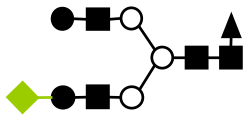
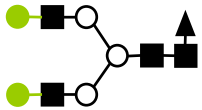
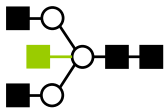
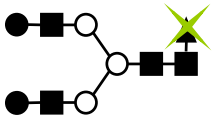
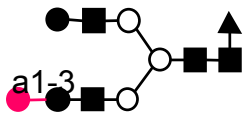



Source: Roche



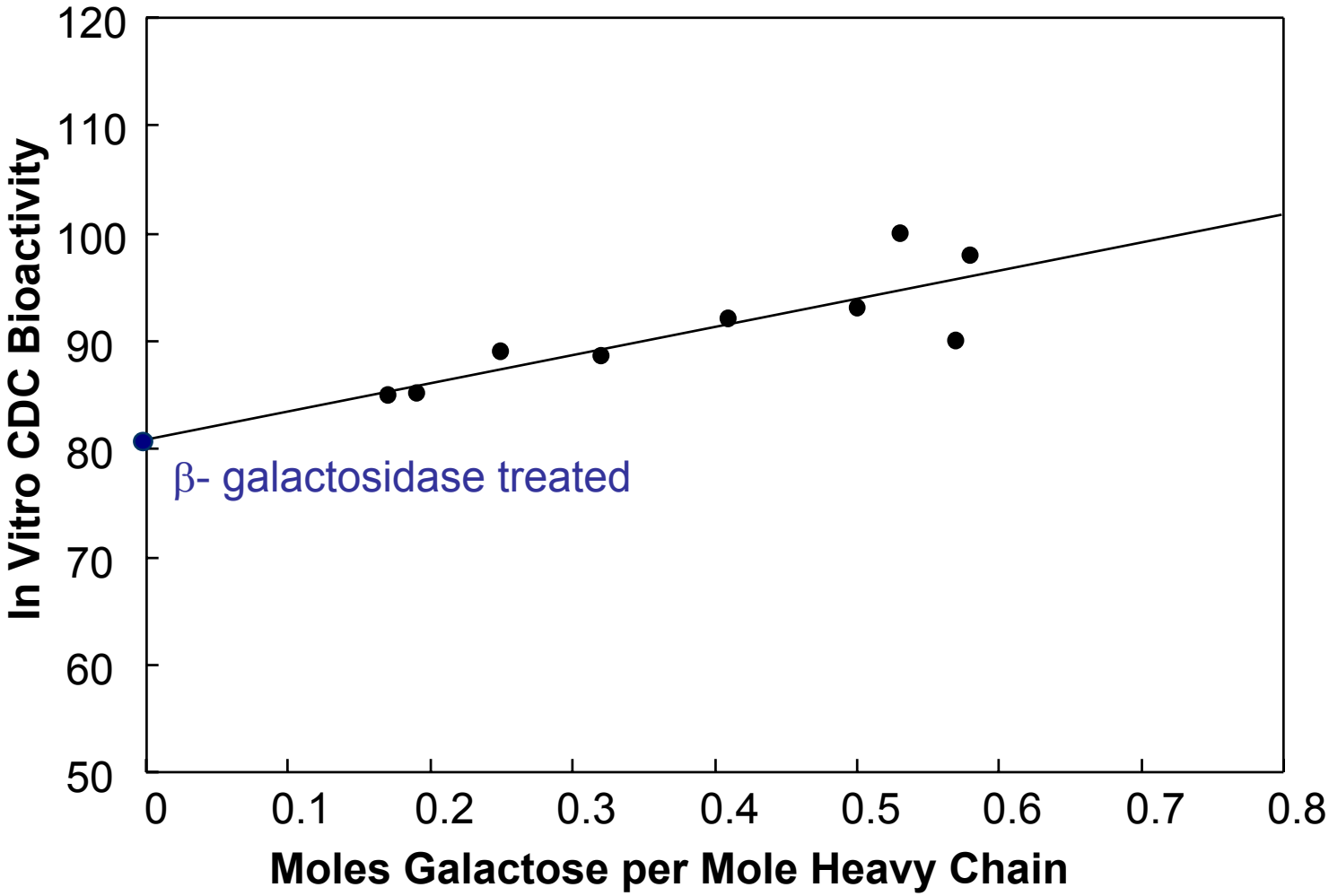

Source: R. Garnick (2008)
Biogenerics 2008 Conference

# Not only Fucosylation is important

| | | |
|---|---|---|
| GlcNAc/ Mannose |  | Ligand for Mannose Binding Protein → complement activation (Malhotra *et al*., Nat. Med. 1995) |
| Sialic acid |  | Suppression of ADCC (anti-inflammatory activity) (Kaneko *et al*., Science 2006) |
| Galactose |  | Placental transport (Kibe *et al*., J. Clin. Biochem. Nutr. 1996) |
| bisecting GlcNAc |  | Prevents core fucosylation → enhanced ADCC (Umaña *et al*., Nat. Biotech. 1999) |
| absence of core Fucose |  | Enhanced ADCC (Okazaki *et al*., J. Mol. Biol. 2004) |
| α(1-3)-Gal |  | Non-human/antigenic (Cooper, Xenotransplantation 1998) |

Source: H. Kettenberger, Roche

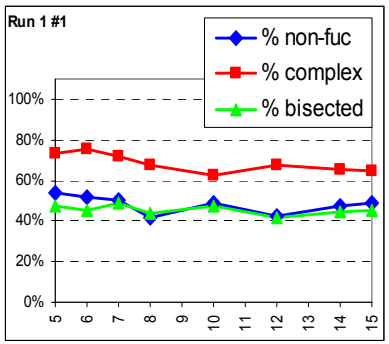
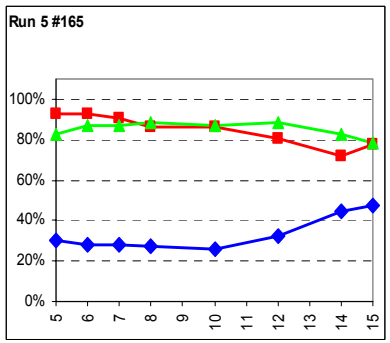
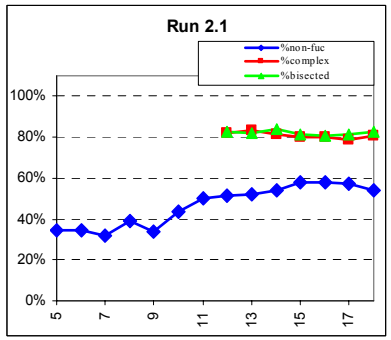
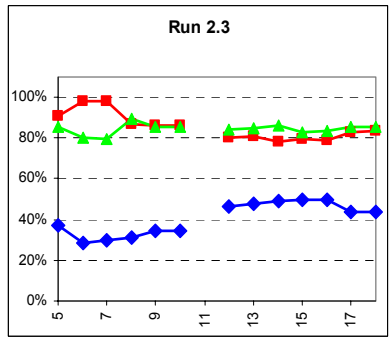
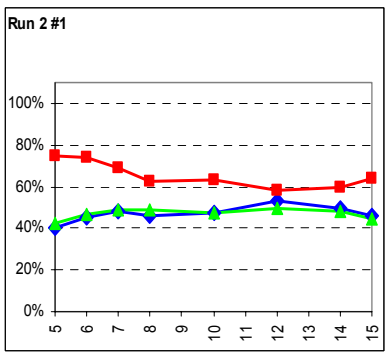
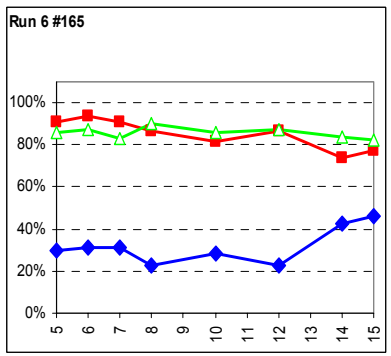
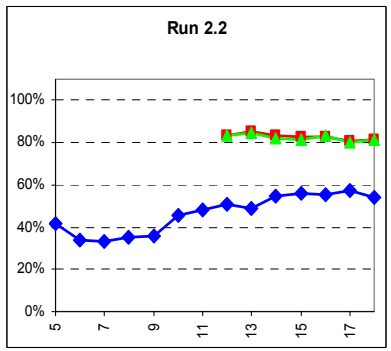
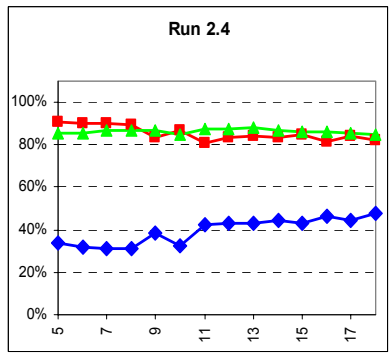
# Correlation of Bioactivity and Galactose Content in Rituximab




Source: Genentech, Inc.

# The Glycopattern depends on the Host Cell and on the Fermentation Conditions

Level of a-fucose during 10-l-lab scale fermentation of a glycoengineered antibody

| Clone 1, 1 X GF | Clone 165, 1 X GF | Clone 165, 1 X GF | Clone 165, 2 X GF |
|---|---|---|---|
|  |  |  |  |
|  |  |  |  |



Source: Roche

# Up to 30% of native human IgGs contain Glycosylation in variable Regions of LC and HC

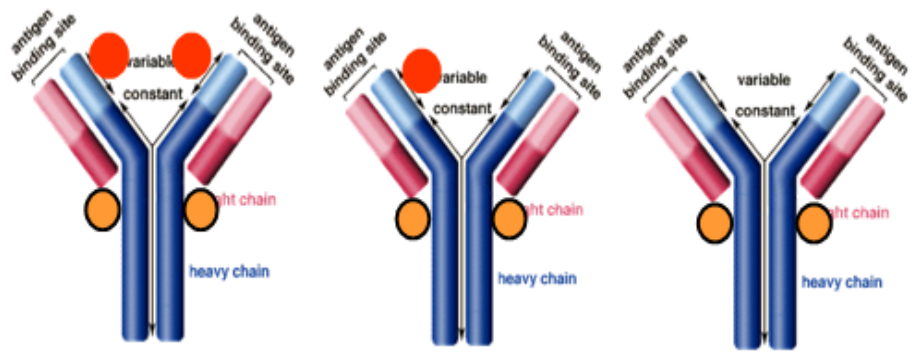
• Mostly not germline encoded; created by somatic mutation. May also be created in mAbs obtained by **phage display** technology.

• **Occupation** ranges from (almost) complete to low percent, depending on glycosylation sequon, accessibility, and **process conditions** (expression system, cell line, fermentation process).

• Different glycan types may be present → **microheterogeneity**

• Potentially has significant **influence on functional and pharmacological characteristics** (e.g., ligand binding, stickyness/unspecific binding, clearance rate, and potency) and technical development (consistency of isoform mixture, pK value, stability, comparability assessment).

• If Fab glycosylation sites are present in the reference mAb, **additional effort** required for analytical characterization, documentation and reporting.



# The Relevance of Fab Glycosylation: The Cetuximab Example

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

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ABSTRACT

BACKGROUND

Cetuximab, a chimeric mouse–human IgG1 monoclonal antibody against the epidermal growth factor receptor, is approved for use in colorectal cancer and squamous-cell carcinoma of the head and neck. A high prevalence of hypersensitivity reactions to cetuximab has been reported in some areas of the United States.

METHODS

We analyzed serum samples from four groups of subjects for IgE antibodies against cetuximab: pretreatment samples from 76 case subjects who had been treated with cetuximab at multiple centers, predominantly in Tennessee, Arkansas, and North Carolina; samples from 72 control subjects in Tennessee; samples from 49 control subjects with cancer in northern California; and samples from 341 female control subjects in Boston.

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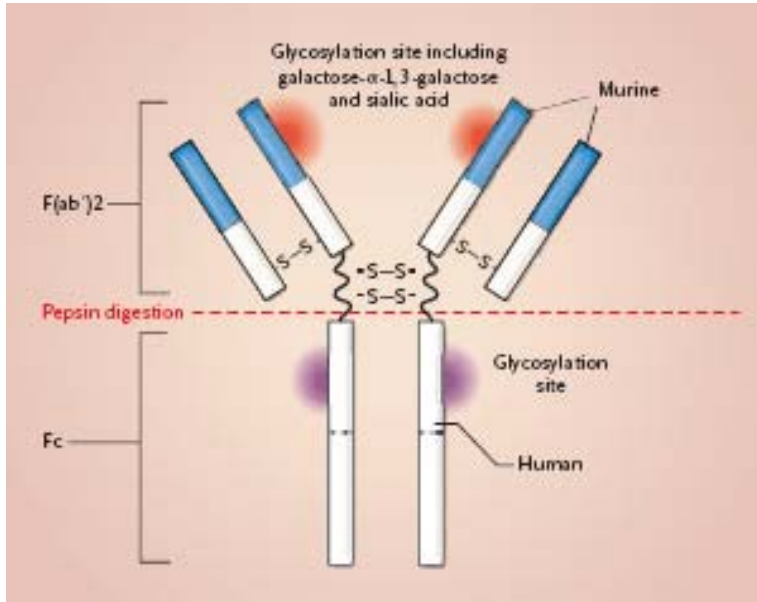



C.H. Chung et al. (2008) NEJM 358, 1109-1117

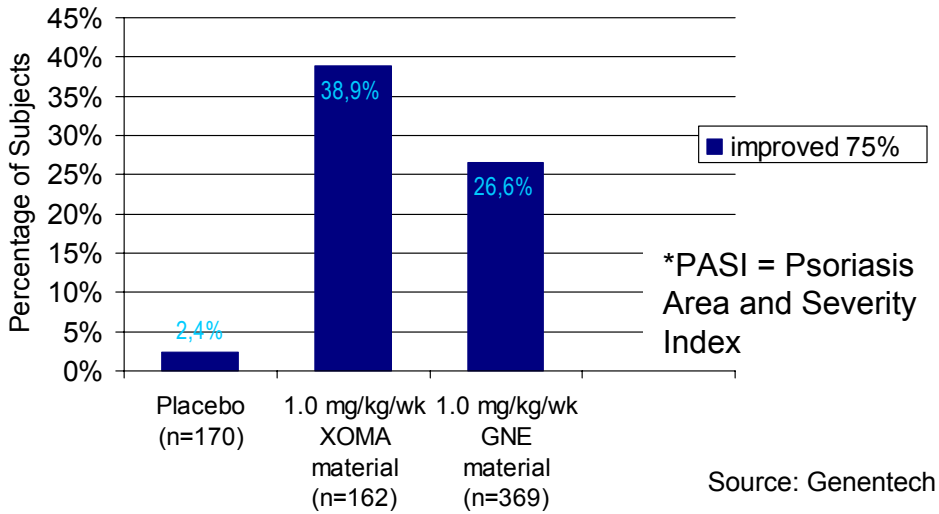
# The Efalizumab Experience

• **Raptiva (efalizumab)** was originally manufactured by XOMA and used in the Phase I/II trials and up to Phase III. Manufacturing was transferred to Genentech with full information. **Analytical and formulation differences** expected to be inconsequential were observed.

• In a **bioequivalence study**, differences in PK parameters were observed:

| | Xoma | GNE | Ratio (G:X) |
|---|---|---|---|
| $AUC_{inf}$ | 27.9 | 36.9 | 1.324 |
| $AUC_t$ | 26.9 | 35.6 | 1.324 |
| $C_{max}$ | 3.59 | 4.22 | 1.175 |

• A trend for **lower PASI* Response to Efalizumab** was found despite higher peripheral drug concentrations




Source: Genentech

• Based on the PK data, one could imagine that administering ~70% of the dose of the Genentech antibody would have similar effects to the XOMA antibody. However, because of the **unpredictable nature of these PK changes** an additional Phase III study was performed to determine the safety and efficacy of the Genentech material.

Changes that were believed not to affect the **properties of the protein** resulted in **clear differences in pharmacokinetics**.
Given the complexity of therapeutic proteins, the **impact** of changes in pharmacokinetics (and probably pharmacodynamics) on **safety and efficacy** can often not be predicted reliably.

# Compendial Methods may fail to reveal Variants with changed Biopotency and Receptor Binding

## Heterogeneity of Commercial Recombinant Human Growth Hormone (r-hGH) Preparations Containing a Thioether Variant

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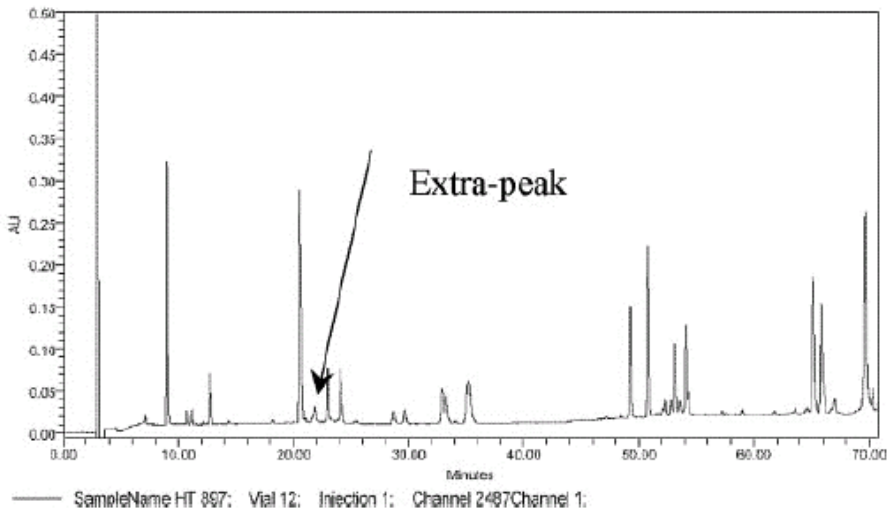


"The compendial methods cover a comprehensive range of degradation forms which take into account the knowledge of the molecular characteristics up to this point in time. However, … these methods **may not be sufficient to appropriately detect the thioether variant**, even if it is present at high levels, and that further powerful methods need to be employed."

Source: M. Lispi et al. (2009) J. Pharm. Sci., DOI 10.1002/jps