Production and Control of Biosimilars versus Innovators

Antonio da Silva, Head Preclinical Development
Hexal / Sandoz Biopharmaceuticals

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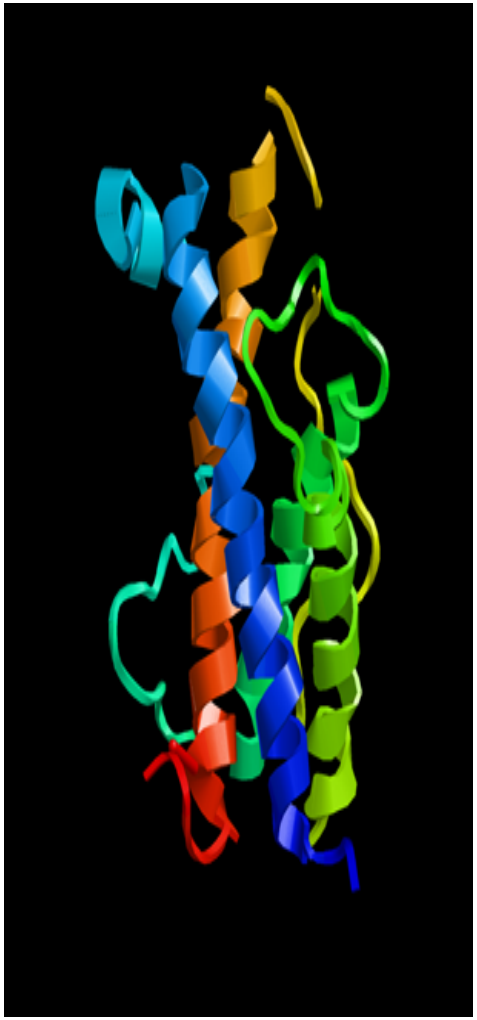
a Novartis company

What is a biosimilar ?



Overview

- **Successor to a biologic** medicine that is already authorized and whose patent has expired
- **Not a simple generic drug** due to complexity, size, structure and manufacturing (not the same)
- Due to complexity, **generics drug approval pathway is not appropriate**

Regulatory definition

- A biologic approved via a **stringent regulatory defined pathway demonstrating comparability**
- **Similar Biological Medicinal Products (Biosimilar) is an EMA regulatory term, not biogenerics** (since 2006)
- **Biosimilar** only applies to products developed **in highly-regulated markets** (no „Me-Too“ or “Copy drug”)
- Regulatory pathways in US being developed under new legislation, increasing interest in multiple countries
- **Same indications** as the **reference product is approved**

Comparability approach

- **Highly analogous structure** to original product (via robust analytical characterization)
- **Comparable quality, safety (incl. immunogenicity) and efficacy** to reference drug to be shown via pre-clinical tests and clinical trials (Ph I & III)

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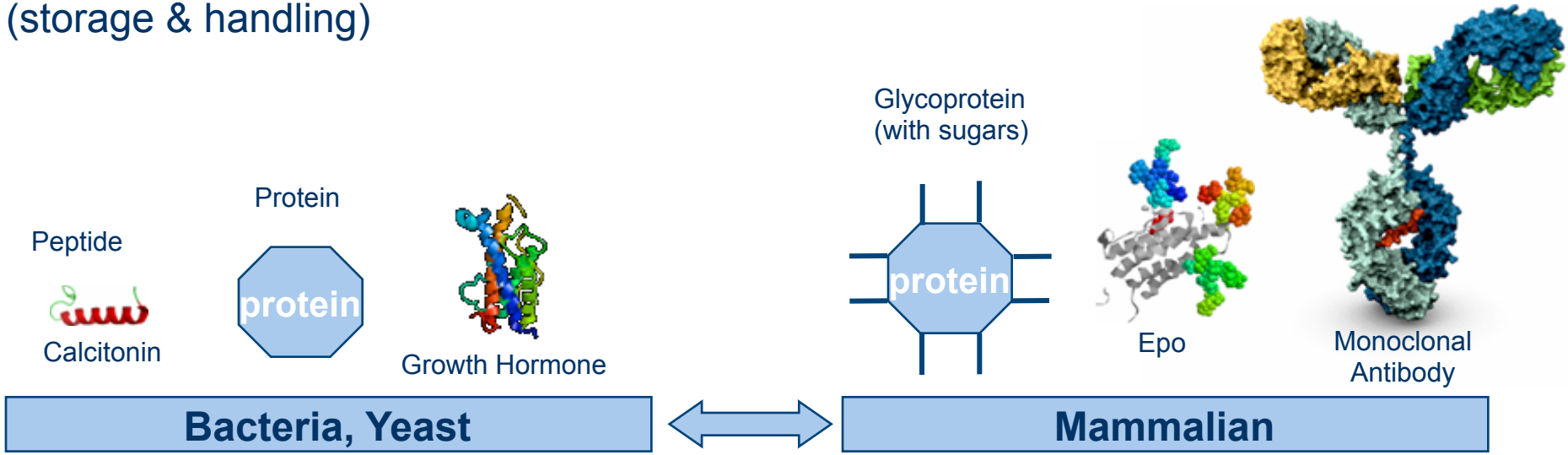
Biologic/Biosimilar manufacturing : Key differences from a small molucule development

- Manipulating host cells (bacteria, yeasts, mammalian) to produce recombinant proteins (develop host cell & establish cell bank)
- Growing them under controlled conditions in steel tanks (fermentation in bioreactors) triggering product accumulation
- Extracting, renaturing (refolding), purifying = generating the drug substance
- Comprehensive characterization and comparability exercise (for biosimilars)
- Formulating to a stable, sellable finished drug product with a convenient device (storage & handling)



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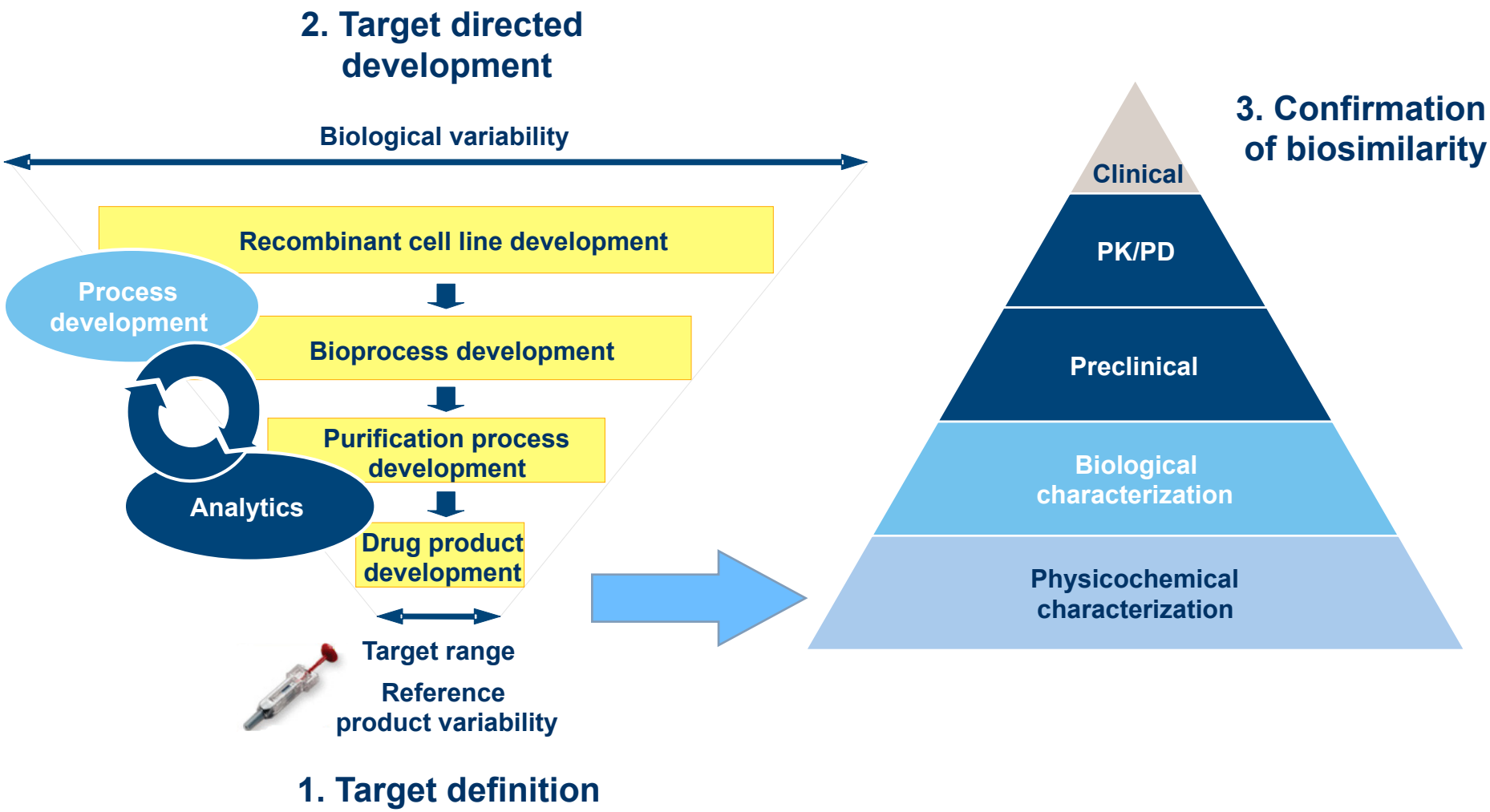
Biosimilars must be systematically engineered to match the reference products



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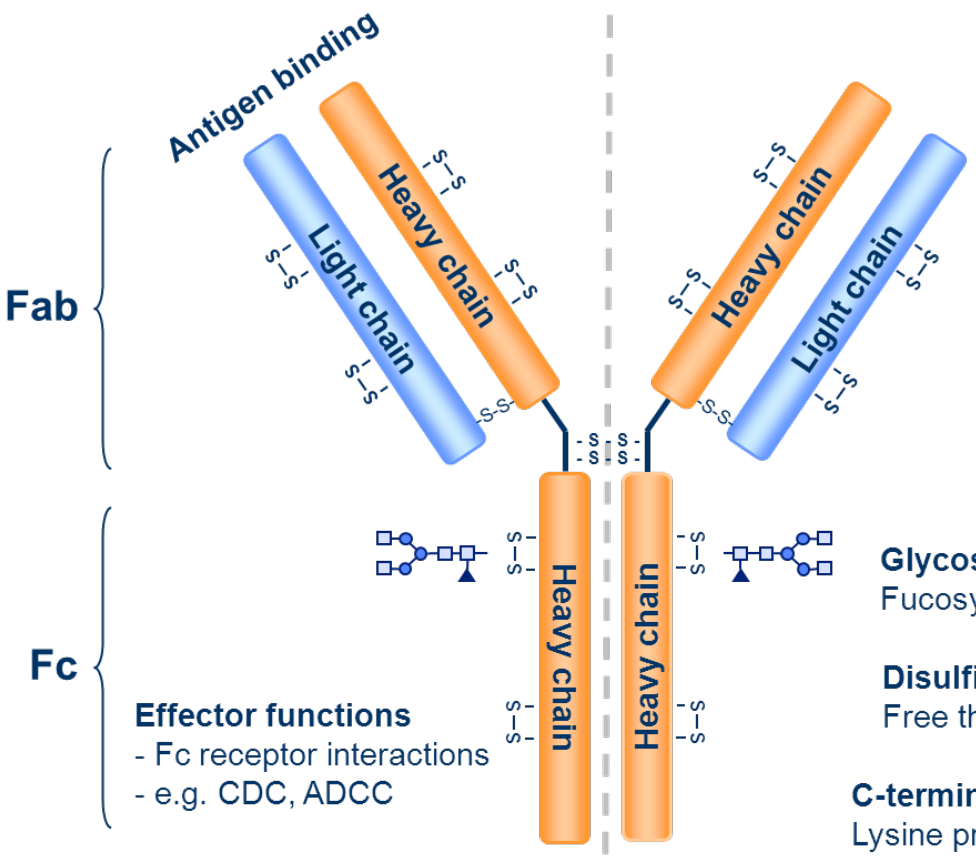
Physicochemical and biological characteristics of a IgG1 monoclonal antibody

Biological characteristics



Physicochemical characteristics

N-Terminal heterogeneity
Pyroglutamate formation
Other modifications

Amino acid modifications
Deamidation, Oxidation, Glycation, Isomerization

Fragmentation
Cleavage in hinge region, Asp-Pro

Aggregation

Glycosylation
Fucosylation, Sialylation

Disulfide Bonds
Free thiols, disulfide shuffling, thioether

C-terminal heterogeneity
Lysine processing, Proline amidation

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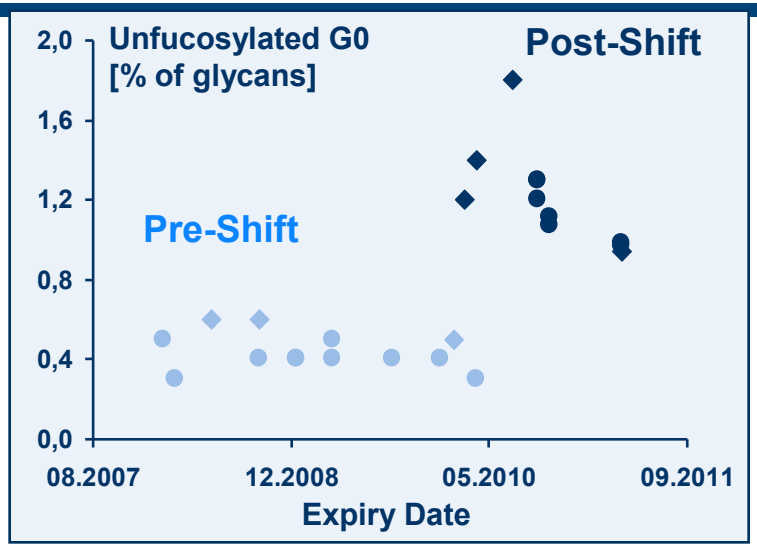
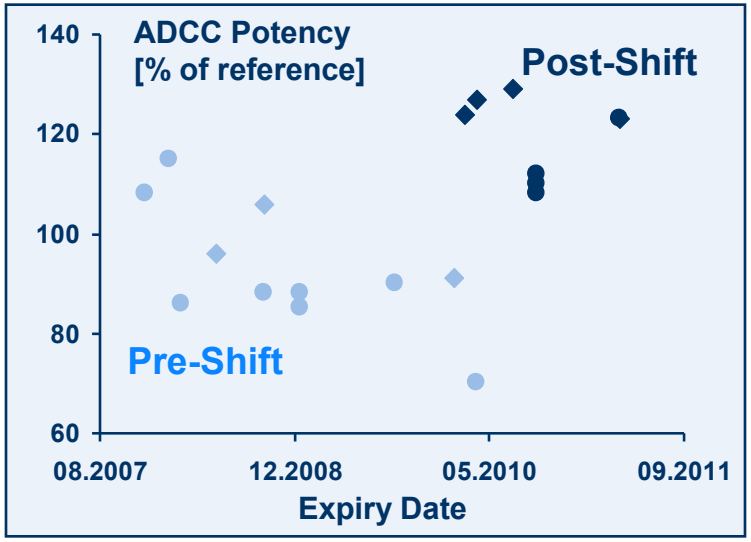
Understanding the target: Variability is significant in reference biologics – antibody example





CORRESPONDENCE

Acceptable changes in quality attributes of glycosylated biopharmaceuticals

Schiestl, M. *et al., Nature Biotechnology* **29**, 310-312, 2011)

- Monitoring batches of an **approved mAb** revealed a **shift in quality**
  - Shift in glycosylation (structure) pattern results in **different potency** in cell-based assays (function)
- Indication of a **change in** the **manufacturing process**
- Such **shifts** observed **in several original products**
  - Products found to be **equally safe and effective post-shift** by regulators (EMA, FDA)

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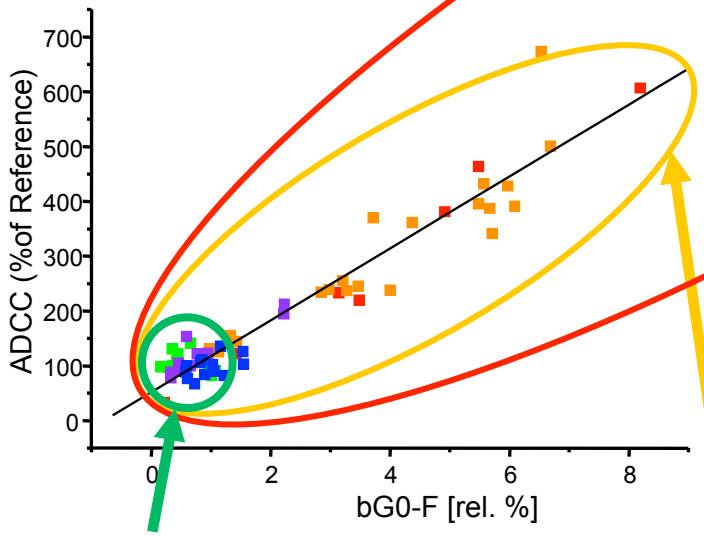
Defining the target: Variability in reference biologic defines very narrow goal posts for biosimilarity



Variability of reference product

Variability observed during cell line development

Biologically possible variability

Very narrow goalposts for biosimilar

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Structure: High resolution, orthogonality and redundancy in analytical characterization provide full understanding

Attributes:

- Primary structure
  - Mass
- Disulfide bridging
  - Free cysteines
- Thioether bridging
  - Higher order structure
- N- and C-terminal heterogeneity
  - Glycosylation (isoforms, sialic acids, NGNA, fucosylation, alpha gal, site specific)
  - Glycation
- Fragmentation
  - Oxidation
  - Deamidation
  - Aggregation

Proteins can be well characterized at least up to the complexity of monoclonal antibodies

- Primary structure determined from recombinant DNA sequence and fully accessible to analytical verification
- Set of orthogonal analytical methods available to characterize the identity and amount of related variants with high sensitivity
- Glycosylation profile can be comprehensively determined with regard to identity and content of individual glycans with high sensitivity
- Accurate and relevant bioassays for pivotal biological functions available

Methods e.g.:

- MS (ESI, MALDI-TOF/TOF, MS/MS)
- Peptide mapping
  - Ellman ‘s
  - CGE
  - SDS-PAGE
  - CD
- H-D exchange
  - FT-IR
  - HPLC
  - HPAEC
  - IEF
- 2AB NP-HPLC
  - SE-HPLC
  - FFF
  - AUC
  - DLS
  - MALLS

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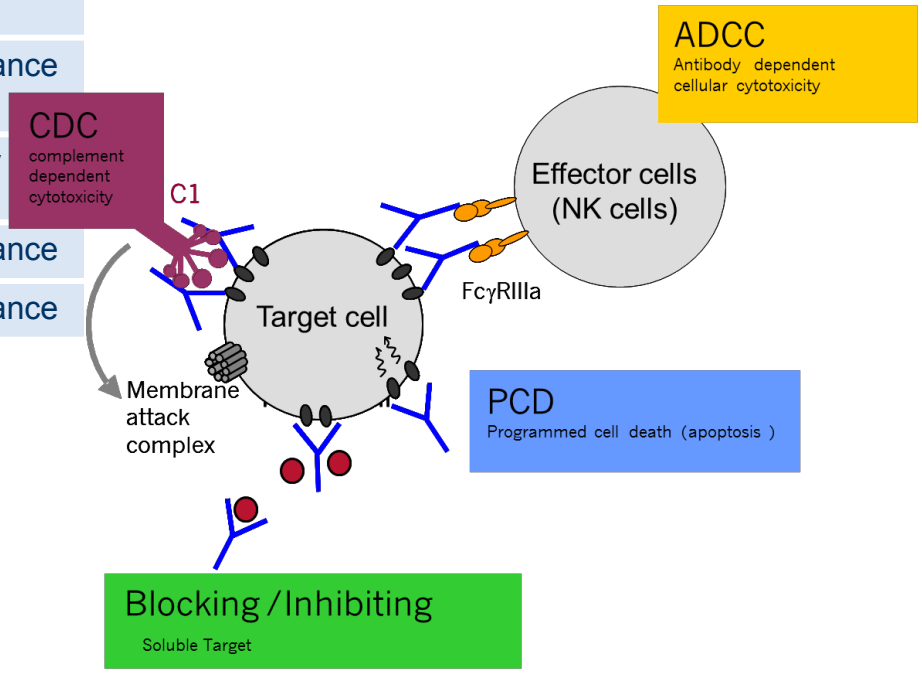
Function: Broad range of sensitive biological, biophysical and immunological assays provides full understanding

Attribute	Functional element	Assay	Formats
Binding to target	Fab	Binding assay	Cellular binding assay Surface plasmon resonance (Biacore®)
Programmed cell death	Fab	Apoptosis assay	Cell based apoptosis assay
CDC	Fab and Fc	CDC assay	Cell based CDC assay
	Fc	C1q binding	Surface plasmon resonance (Biacore®)
ADCC	Fab and Fc	ADCC assay	Cell based ADCC assay
	Fc	FcγR binding	Surface plasmon resonance
PK	Fc	FcRn binding	Surface plasmon resonance



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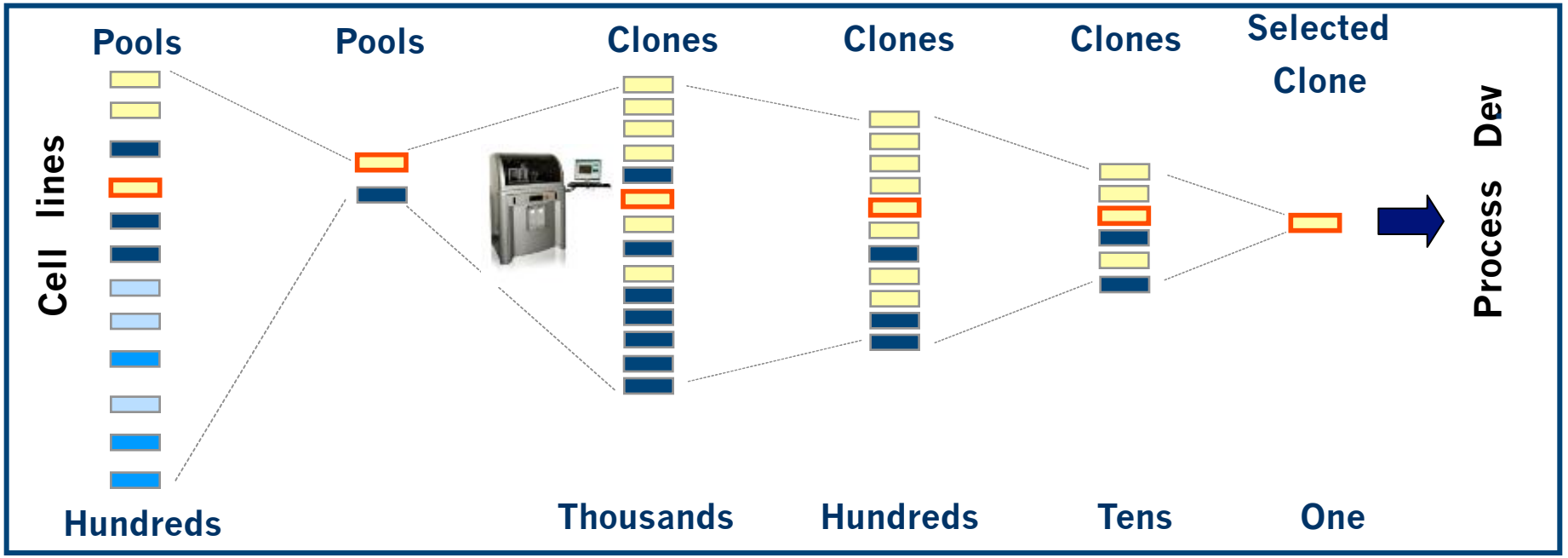
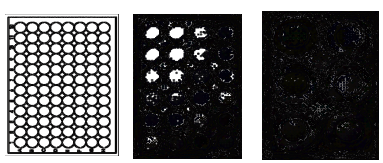
Cell line development: An elaborate, target-directed multi-stage process





96/24/6 well plates

Shake flask

Lab-scale bioreactor

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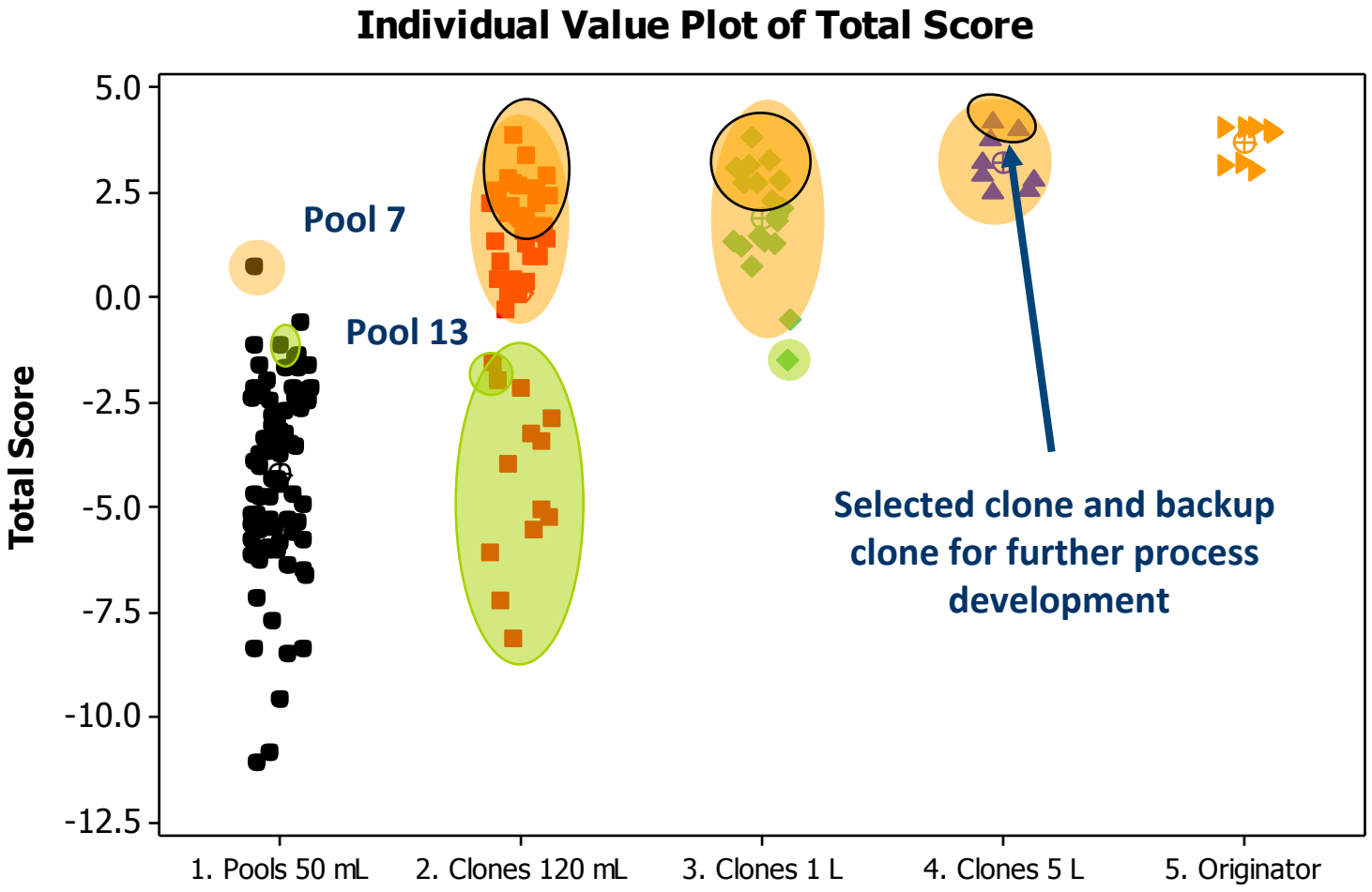
Cell line development: Multiple selection rounds required to hit the target ("evolution in the test tube")



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Understanding the criticality of the quality attributes is essential

- Risk assessment for ranking and prioritizing quality attributes
  - General concept described in A-MAb case study (Tool #1)

Criticality Score = f(Impact,Uncertainty)

e.g.: Criticality Score = Impact x Uncertainty (A-MAb)

	Impact	Uncertainty	Criticality Score
	Known or potential consequences on safety and efficacy, considering: • Biological activity • PK/PD • Immunogenicity • Safety (Toxicity)	Relevance of information e.g. literature prior knowledge *in vitro* preclinical clinical or combination of information	Quantitative measure for an attribute's impact on safety and efficacy. Using best possible surrogates for clinical safety and efficacy
Range	**2 (very low) – 20 (very high)**	**1 (very low) – 7 (very high)**	**2 - 140**

Accumulated experience, relevant information, data

e.g. literature, prior & platform knowledge, preclinical and clinical batches,*in vitro* studies, structure-function relationships

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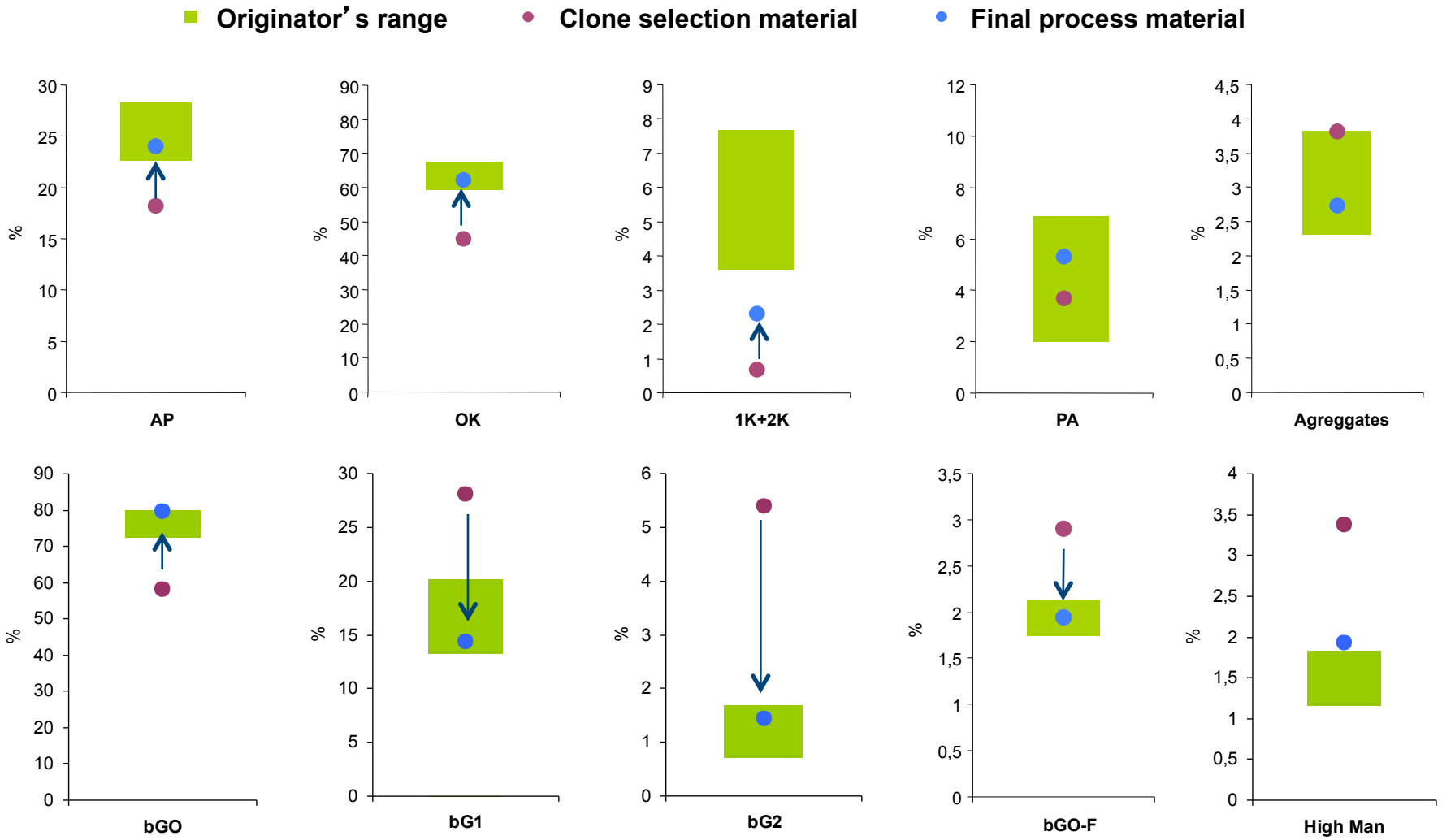
Process development (example): Product quality optimization to originator's profile



Disclaimer: all values are hypothetical

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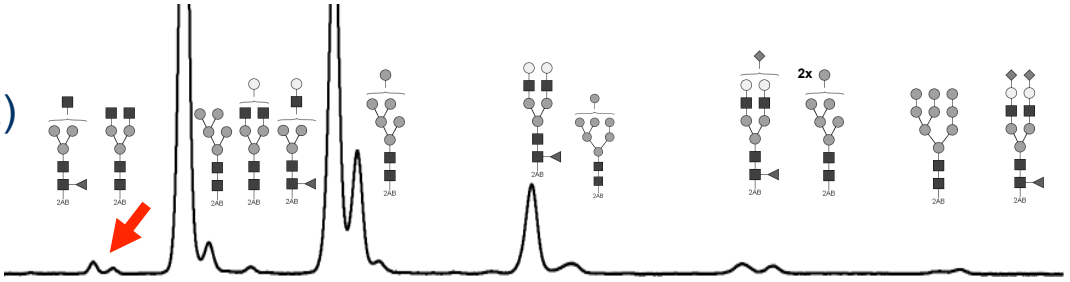
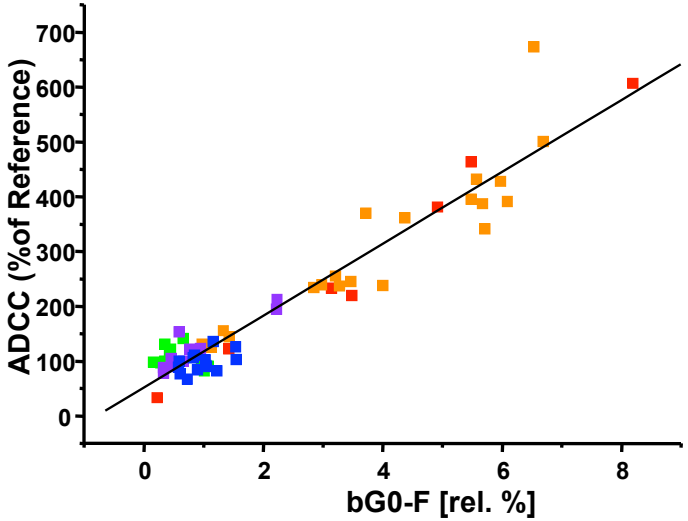
Cell line development case study: Minor glycan structures and ADCC bioactivity – attention to detail is essential...

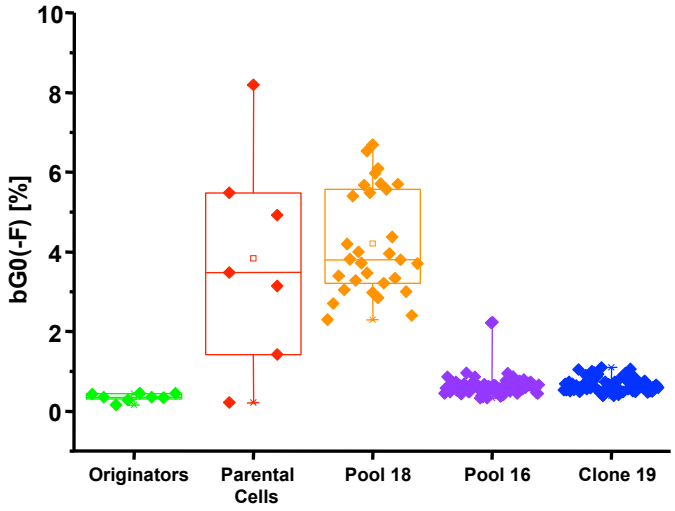
Characterization of mAB glycosylation heterogeneity

High resolution identification and quantification of major (G0,G1,G2) and minor glycan structures (down to a level of 0.1 rel.%)



Targeting ADCC activity and fucosylation by clone selection





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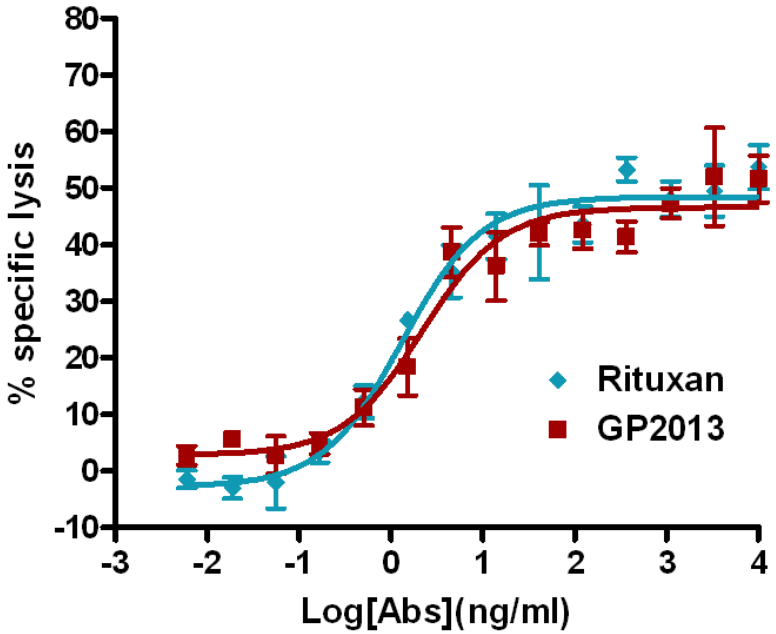
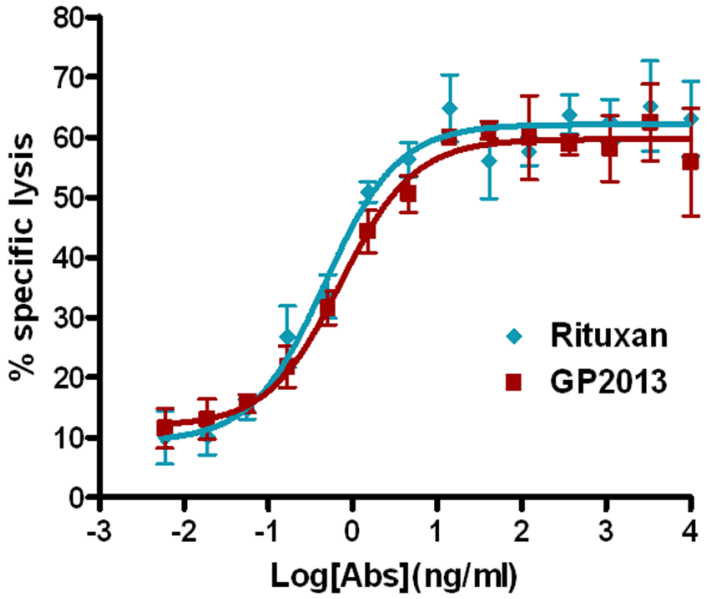
In-vitro comparability: ADCC assays using clinical scale GP2013 (rituximab) drug product

Daudi cell line & fresh effector cells



SU-DHL4 & fresh effector cells



Further cell lines tested:
- Raji
- Z138

√ **ADCC comparable to MabThera® / Rituxan®**

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Conclusions

Today, biologics can be thoroughly characterized and understood both structurally and functionally

The goal posts are set by the variability in the reference product and CQA understanding

Knowledge of how process parameters influence product attributes is used to achieve matching quality

Analytics is more sensitive in detecting differences than clinical studies