

## Abstract

Over 70% of therapeutic antibodies are produced in CHO (Chinese Hamster Ovary) cell systems, prized for their human-like post-translational modifications and proper protein folding. However, their lower transfection efficiency compared to HEK cells makes them less suitable for early-stage drug development, where rapid screening and high-yield transient production are crucial. As a result, initial screenings are often performed in HEK cells before shifting to CHO for large-scale production—a transition that introduces challenges such as expression re-optimization, productivity loss, and potential misselection of antibody candidates. To streamline this process, ProteoGenix has developed XtenCHO™ Race, a CHO-K1-derived transient expression system that supports high-throughput small- to large-scale antibody production. Surpassing ExpiCHO™ in performance (as shown in this comparative study), XtenCHO Race™ maximizes transient yields while ensuring a seamless transition to stable cell line development, maintaining consistency from early research to industrial manufacturing.

## Cell lines engineering

Starting cell line: Wild Type CHO-K1

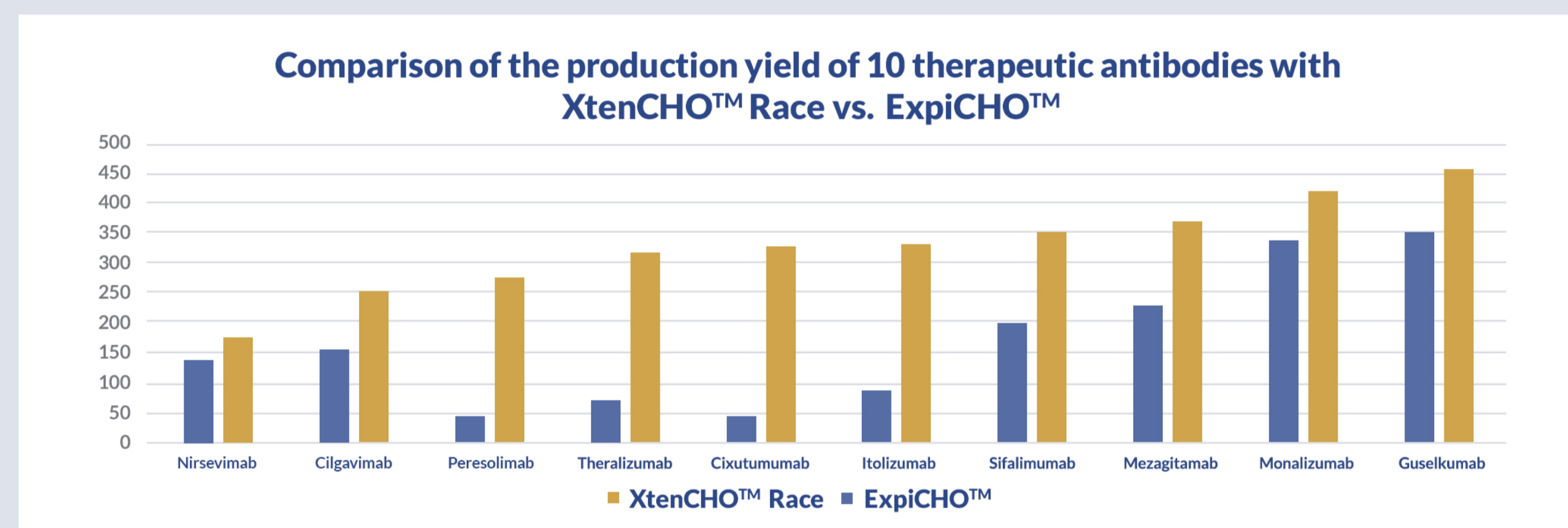
Genetic modification(s) [KO and/or KI]

XtenCHO™ Race Cells

Extremely high expression levels

## XtenCHO™ Race cells vs. ExpiCHO™ - Production yield comparison

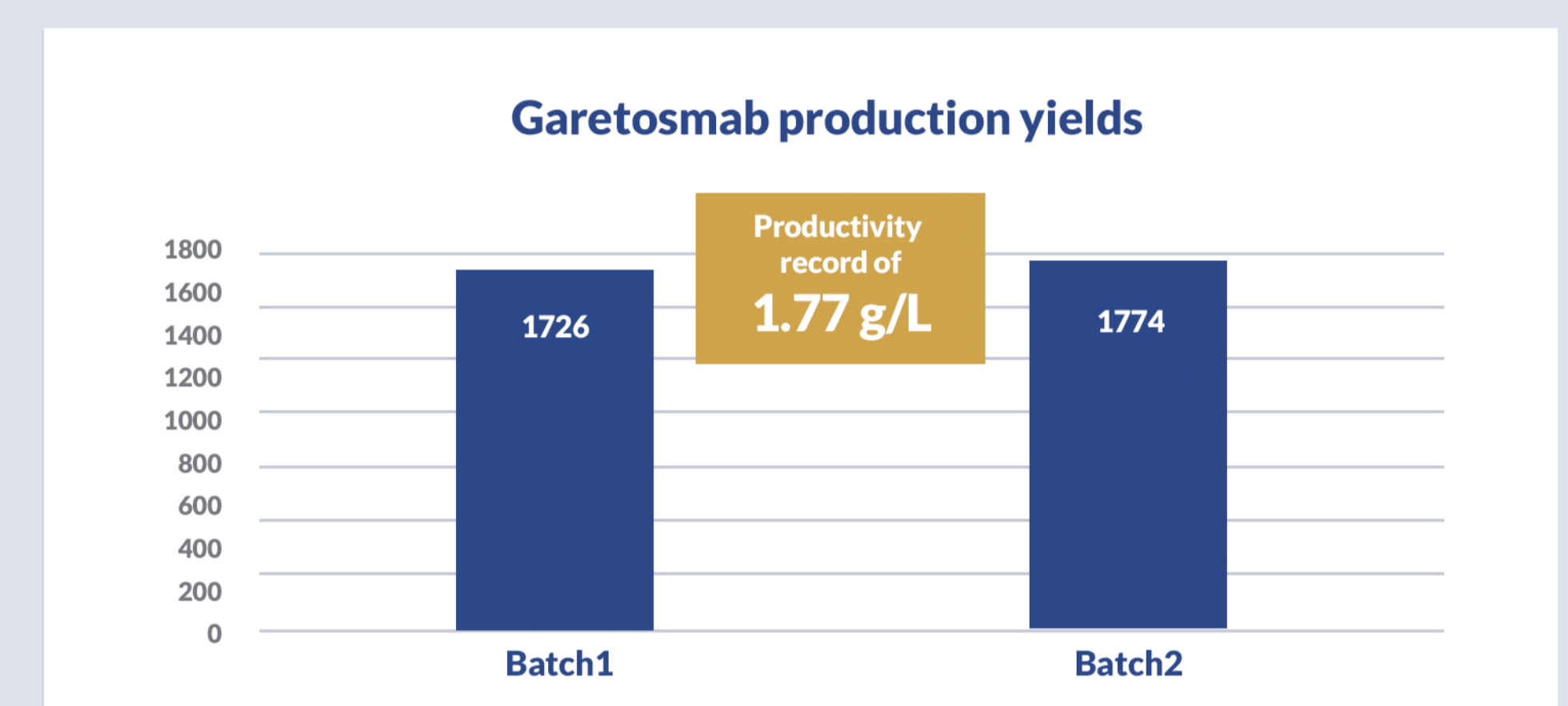
Therapeutic antibodies from several IgG subclasses and isotypes (IgG1 – kappa and lambda, IgG4 - kappa) were produced with XtenCHO™ cells, and in parallel with ExpiCHO™ expression systems. XtenCHO™ Race cells were transfected with ProteoGenix' proprietary TGE system (transfection protocol, transfection reagent and culture medium). ExpiCHO™ cells were transfected according to manufacturer's instructions for each expression system (protocol, reagents and culture medium). Determination of production yields was done with an Octet Red96 system (Protein G biosensor).



→ Production yields obtained with XtenCHO™ Race are twice higher than those obtained with ExpiCHO™

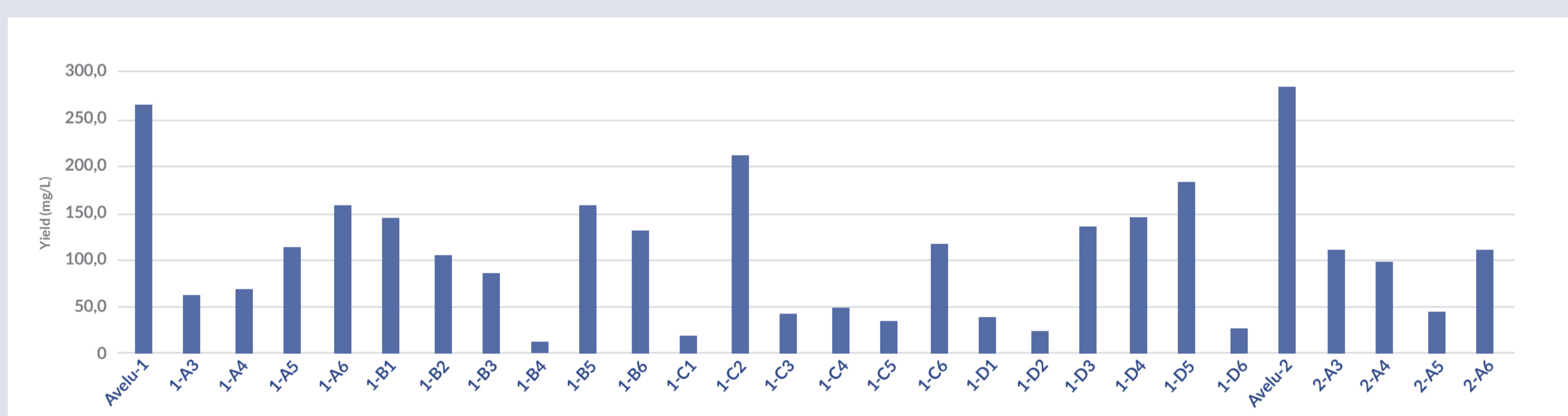
## XtenCHO™ Race production yield record

The therapeutic antibody Garetosmab (IgG4) was produced with XtenCHO Race™ cells. Different expression batches were obtained with variations of transfection and culture conditions in order to optimize the production of this high expressing antibody. Productions were done in standard shaking flasks, without any feeding or booster.



## High-throughput therapeutic antibody production

The XtenCHO™ Race kit, combined with our protocol, has been tested for high-throughput antibody production. The production of 26 recombinant antibodies (recAbs) was carried out in parallel on a 24-well plate. Production yields were determined using an Octet Red96 system (Protein G biosensor).

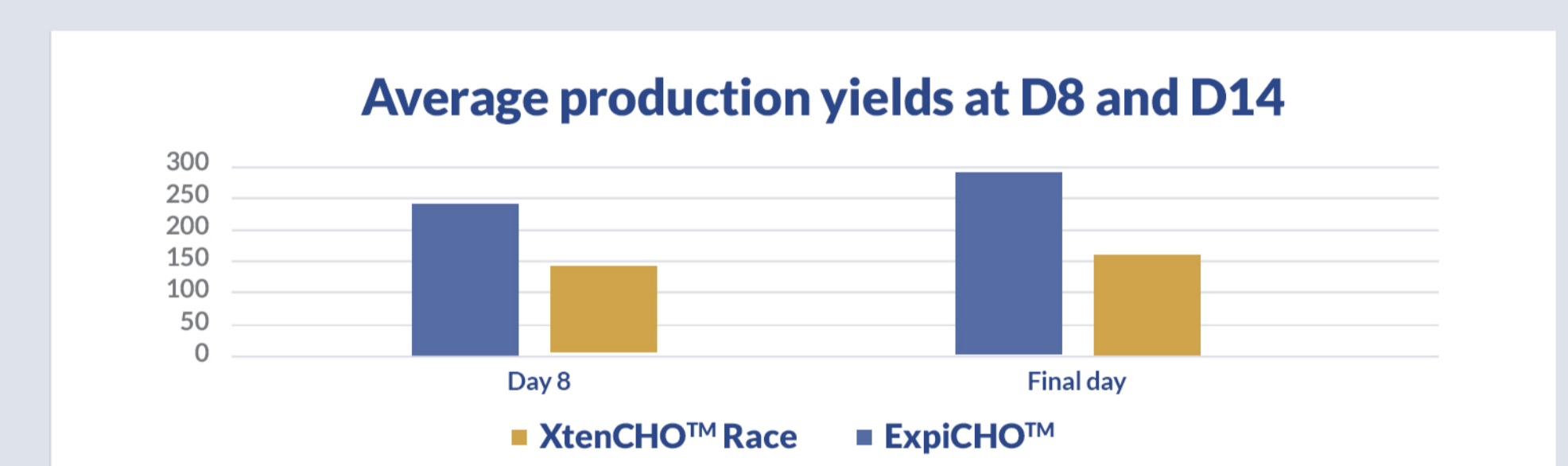


→ Expression yields comprised between 10 and 280mg/L (average yield: 107 mg/L)

→ High expression of all humanized variants - Allows to perform crucial in vitro and in vivo functional tests rapidly

## XtenCHO™ Race vs. ExpiCHO™ - Production yields at different times

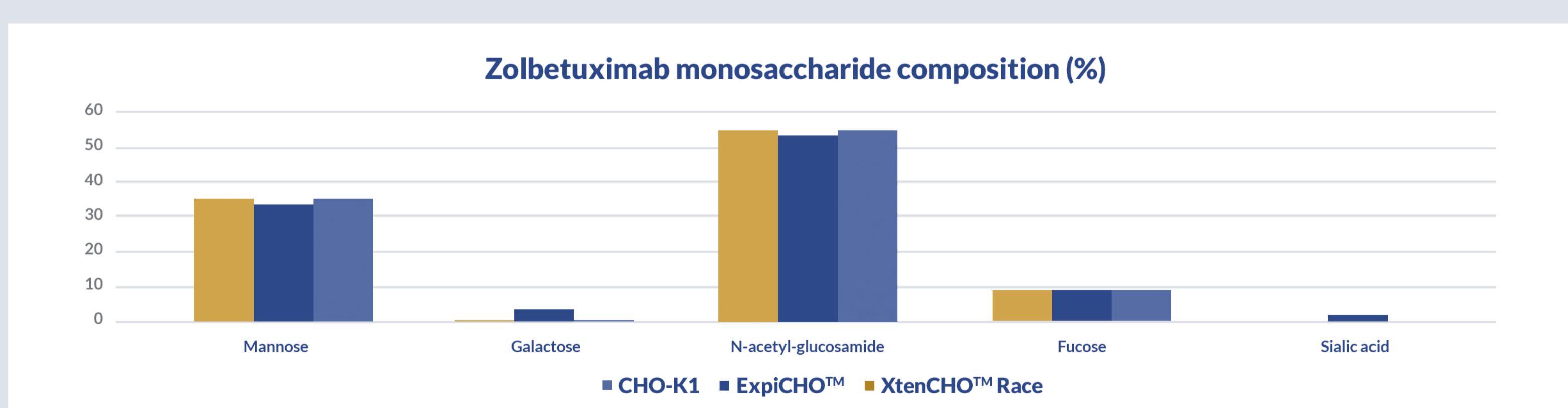
The production yields of XtenCHO™ Race were compared to those of ExpiCHO™ at different time points (D8 and D14) for 2 therapeutic antibodies (Olaratumab and Cixutumumab). Each expression system was used according to the manufacturer's instructions. Production yields were determined using an Octet Red96 system (Protein G biosensor).



→ XtenCHO™ Race overperforms ExpiCHO™ production yields by 70% at D8 and 80% at final day

## XtenCHO™ Race vs. ExpiCHO™ - Post-translational modifications

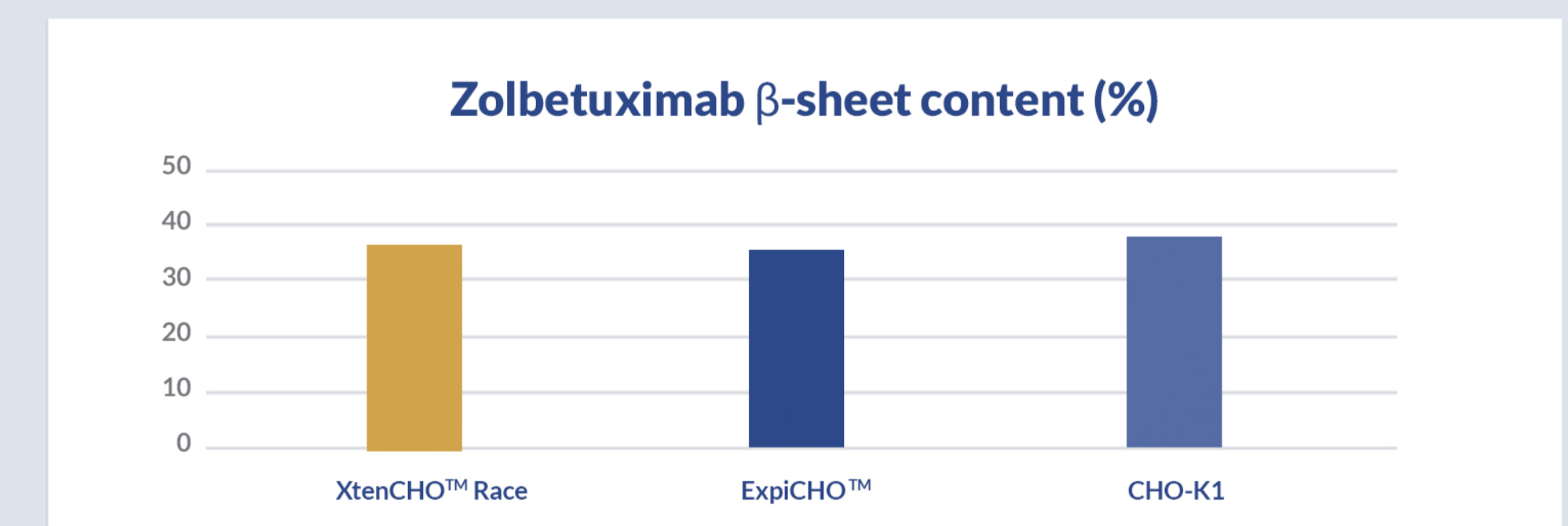
Monosaccharide composition was additionally evaluated with Fourier Transform Infrared Spectroscopy (FTIR) for Zolbetuximab (IgG1) produced with the TGE systems XtenCHO™ Race and ExpiCHO™, and the stable expression system CHO-K1. The composition was found to be similar between Zolbetuximab's variants produced in TGE and the stable antibody production system. Interestingly, slight differences were found in Galactose, N-acetylglucosamine and Sialic acid composition, with XtenCHO Race™ presenting more comparable profiles to those measured in CHO-K1, the gold standard for therapeutic antibody production.



→ Similar post translational modifications found in XtenCHO™ Race, ExpiCHO™ and CHO-K1

## XtenCHO™ Race vs. ExpiCHO™ - Secondary structure prediction

The secondary structure ( $\alpha$ -helix and  $\beta$ -sheet) was evaluated with FTIR for the therapeutic antibody Zolbetuximab (IgG1) produced in TGE systems (XtenCHO™ Race and ExpiCHO™), and in CHO-K1 (stable expression). No  $\alpha$ -helix content was detected in Zolbetuximab's variants produced in the different systems (data not shown). The  $\beta$ -sheet content was similar between the three systems with a slightly lower content detected in the variant produced by ExpiCHO™.



→ Comparable  $\beta$ -sheet content detected in all expression systems

## CONCLUSION

- New CHO cell line developed by ProteoGenix.
- Outperforms ExpiCHO™ expression systems in its own optimal working conditions.
- High performance cell line for TGE of recombinant antibodies in an animal-free, serum-free, protein-free medium.
- Comparable secondary structure and post-translational modifications to those observed in ExpiCHO™ and CHO-K1 (stable expression system).
- Enables high-throughput production up to mid/large-scale production.
- Allows antibody harvesting as early as day 8.