AbtBioConsult
Supporting biologics CMC development

Will Technological Progress in CLD Enable a Faster Track to the Patient?

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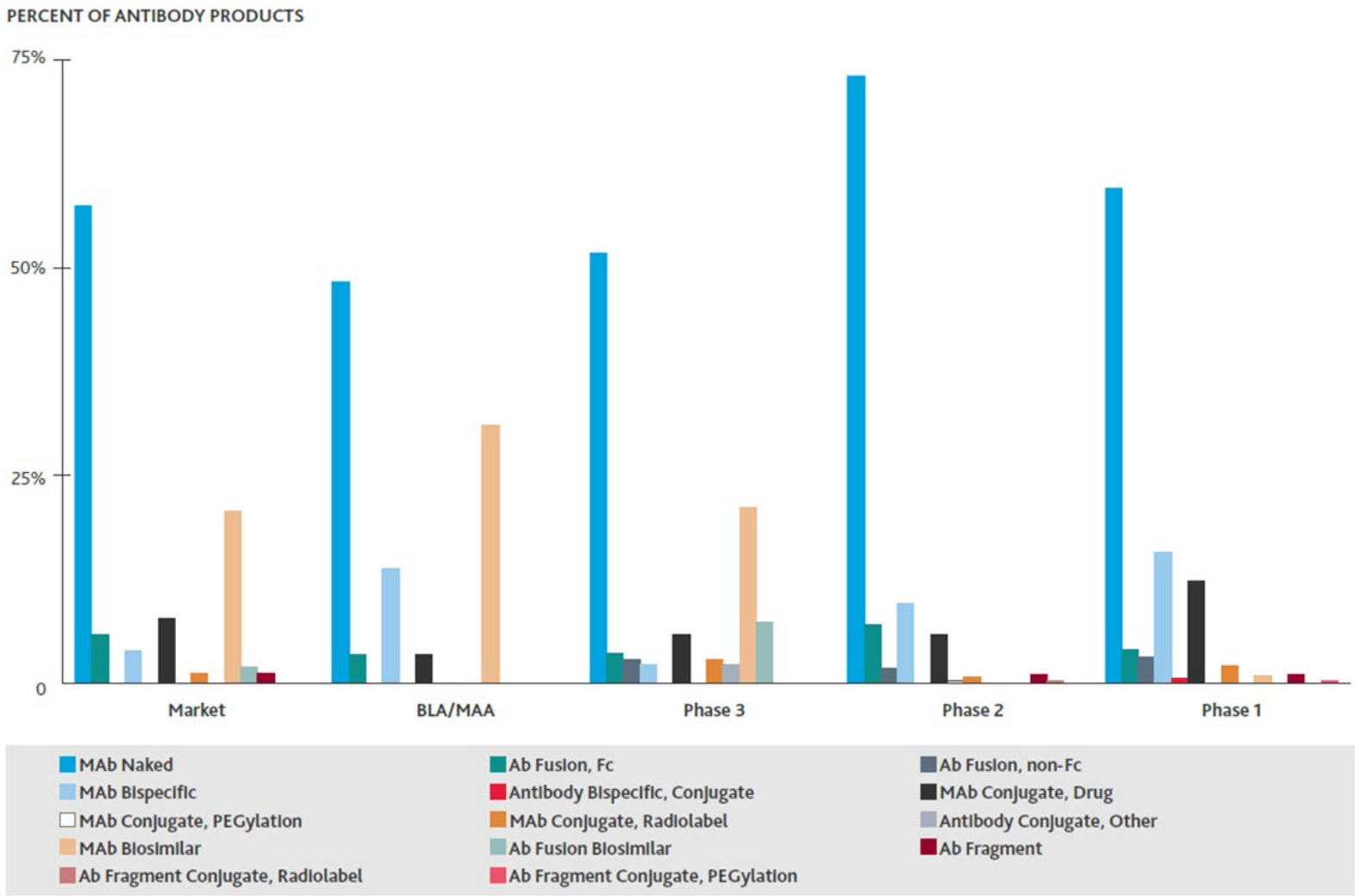
Overview

This talk will review the history of recombinant cell line development and the most current trends also in relation to regulatory acceptance.

Mammalian Biologics Product Distribution by Product Type and Clinical/Market Stage



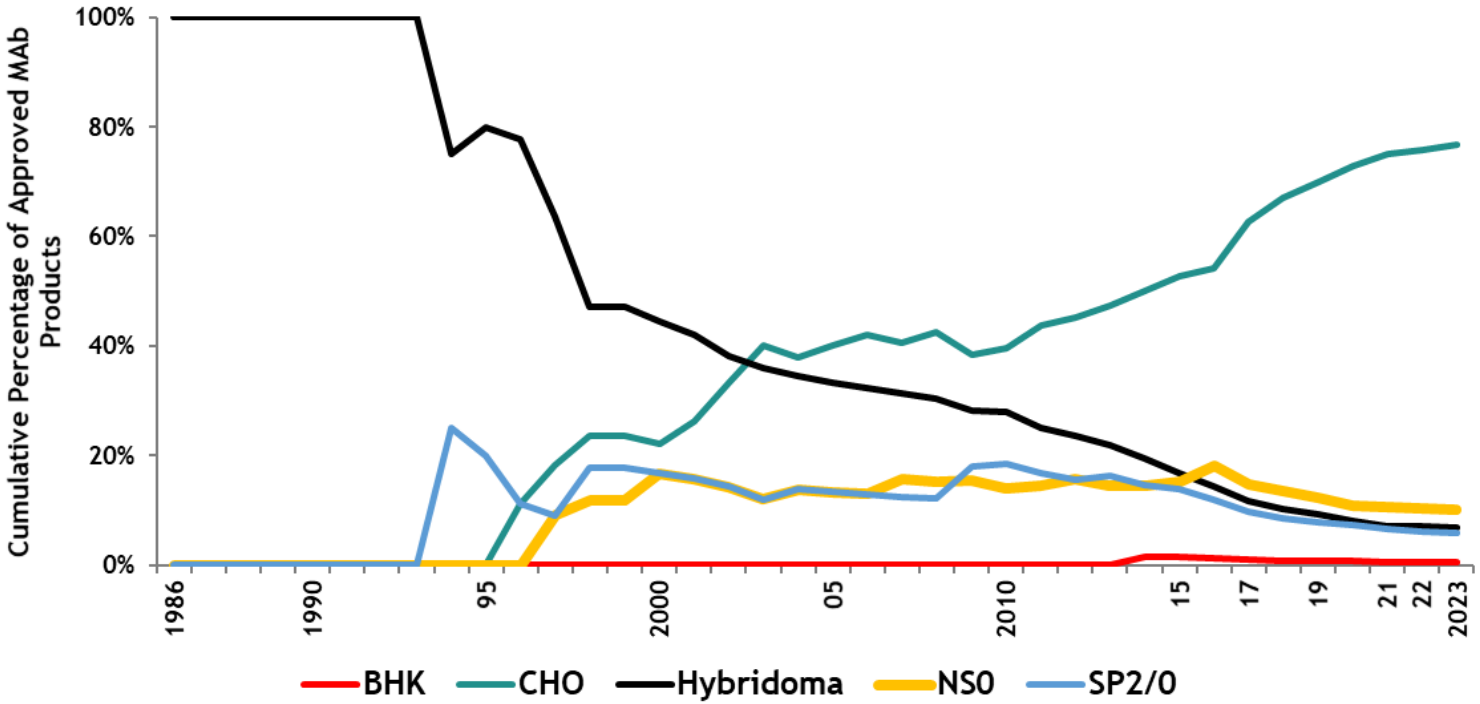
Across the entire pipeline, naked mAbs are the dominant product type:

- Marketed Products: 57%
- BLA/MAA: 48%
- Phase 3: 52%
- Phase 2: 73%
- Phase 1: 60%

Other formats are on the rise

Data and graphs derived from BDO's BPTG bioTRAK® database: https://www.bdo.com/industries/life-sciences/bioprocess-technology

Host Cells for the Production of Antibody Products



- In 2023, CHO cells comprise 77% of all cell lines used to manufacture Commercially approved Mabs
  - NS0 represent 10%
  - Hybridomas 7%
  - SP2/0 6%
  - BHK <1%

Data and graphs derived from BDO's BPTG bioTRAK® database:
https://www.bdo.com/industries/life-sciences/bioprocess-technology

Chinese Hamster Ovary (CHO) History

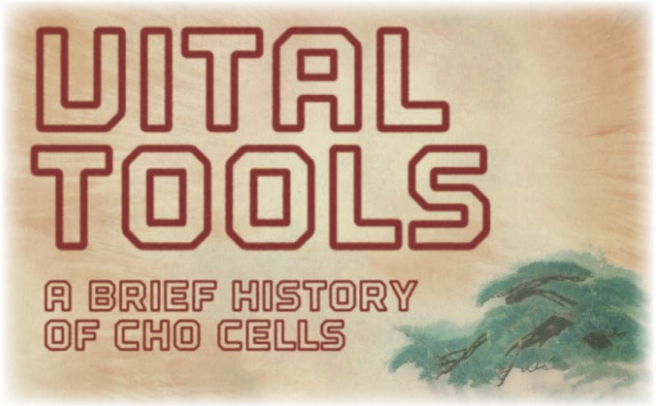
- **Chinese Hamsters** were imported to North America from China during the Chinese civile war in **1948**
- Dr. Theodore Puck established the first CHO cell line in **1957**
- Since 1968, **adherent** CHO cells are available for purchase for example from ATCC (CHO-K1)
- Since 1980, Dr Lawrence Chasin, University of Columbia, New York, distributes **adherent** CHO DG44 and CHO-DXB11 $dhfr^-$
- Other CHO sources, for example ECACC and suppliers in the biopharma field are also available now
- Suspension-adapted CHO cell lines are also available now (CHO-S)

Note:

CHO adaptation from adherent to suspension culture easily takes 2-3 months

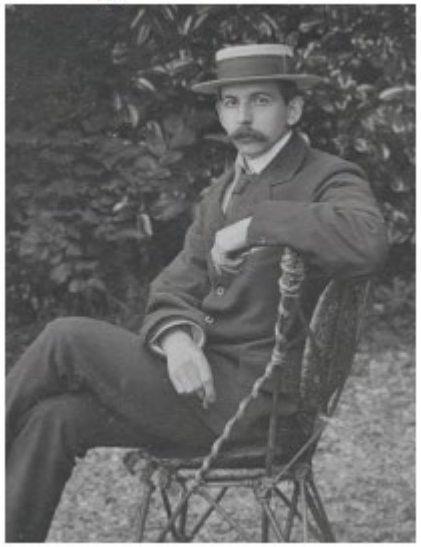
Chinese Hamsters travelled from China to North America during the Chinese Civic War in 1948

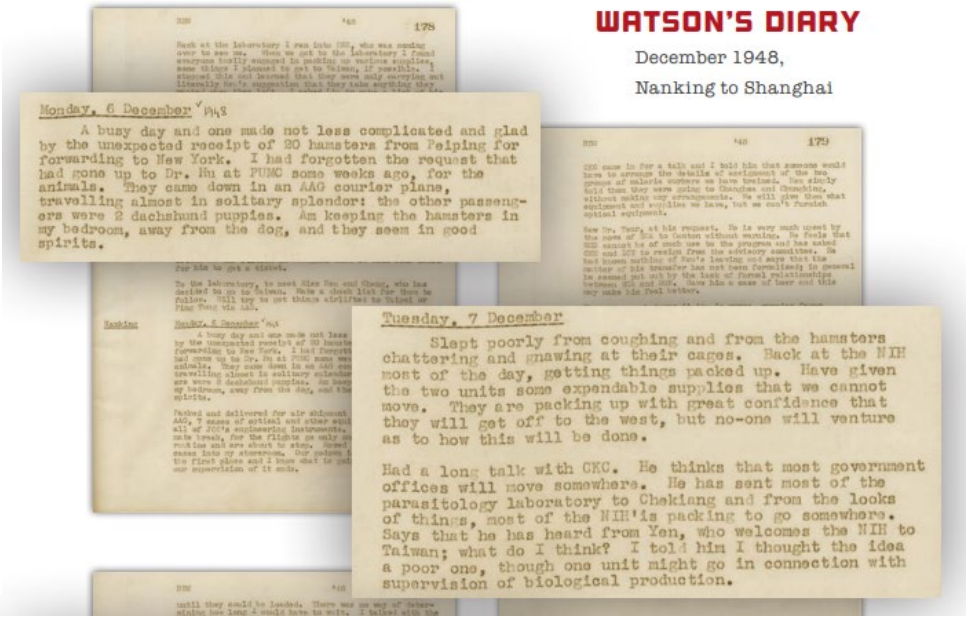
They barely made one of the last Pan-Am flights out of Shanghai before the Maoists claimed victory



http://biomanufacturing.org/uploads/files/547998065159985597-cho-history.pdf

Robert Briggs Watson in the 1920s





Pan-Am routes in the Americas and across the Pacific in 1947

In 1957, Puck learned of the Chinese hamster and its compact genome. He contacted George Yerganian and asked for specimens. Yerganian sent a single adult female, housed in a handmade box with a mesh top. She arrived by railway courier, after riding trains for several days. No one could have predicted how important this single hamster would become in the history of the life sciences, biomedicine, and the biopharmaceutical industry.

Dr. Theodore Puck

Not for use/distribution

CHO Cells are not just CHO Cells

Hamster → CHO → CHO Pro-

Isolated by Tijo & Puck 1958

Proline-dependent mutant Puck et al. 1967

subclones of Pro-

- **CHO-S** — Invitrogen
- **CHO-K1** — Puck et al., 1968, ATCC CCL-61
- **Pro-5** — P. Stanley, Cell 1975, ATCC CRL-1781

Not for use/distribution

- **CHOK1SV** — Lonza
- **DG44** — Urlaub & Chasin 1980
- **DuxB11**
- **Lec mutants** — P. Stanley

Wild type

dhfr -/-

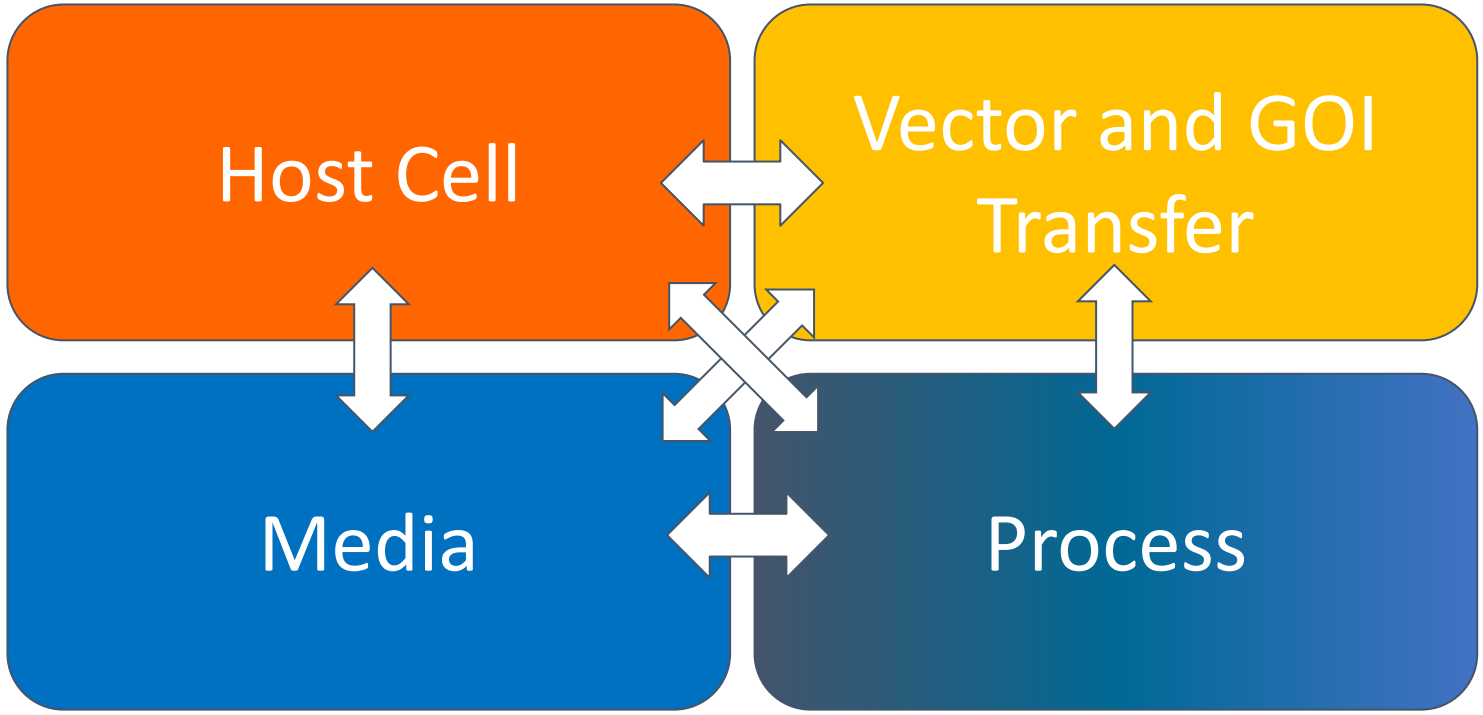
- **HEX1**
- **HEX2**

Boehringer-Ingelheim

- **ECHO** — Symphogen
- **DP12** — Genentech

Most BioPharma and Contract Manufacturers developing biopharmaceuticals have their own (suspension-adapted) CHO host cell as an integral part of their platform

The Four Building Blocks of a Manufacturing Platform



All these four factors are strongly interlinked
Knowledge and experience with your manufacturing platform is key
But even with a well-working platform, variation can be huge

CHO vectors and host cells optimizations enabled platform establishment

Expression vector design

- Promoters, polyA, one vector/two vectors, IRES, selection markers etc…

Host cells

- CHO DG44: MTX selection, optional amplification
- CHO-K1 GS^-: Glutamine synthetase deficient, enabling selection in glutamine-free media
  - CHO GS KO cell lines have since patent expiry been made multiple times, for example via Zinc finger (eg CHOZN), Cre-Lox or Crispr-Cas technologies
  - Individual biopharma and CDMO platform host cells are most often engineered or optimized in other ways
  - Their engineering approaches may not be fully disclosed

Expression platforms

- Tight integration with process and media optimization

Clonality!

Production instability was a concern in early days of CHO based manufacturing

One serious consequence of this instability was FDAs request for proof-of-clonality (~2008)

Two rounds of limited dilution

FACS + imaging

Berkeley light

ClonePix – two rounds

Cell printer + imaging

Need for significant time consuming and labor heavy clone screening and documentation

Robots and automation play an ever increasing role here – but this is expensive!

CLD Documentation Requirements

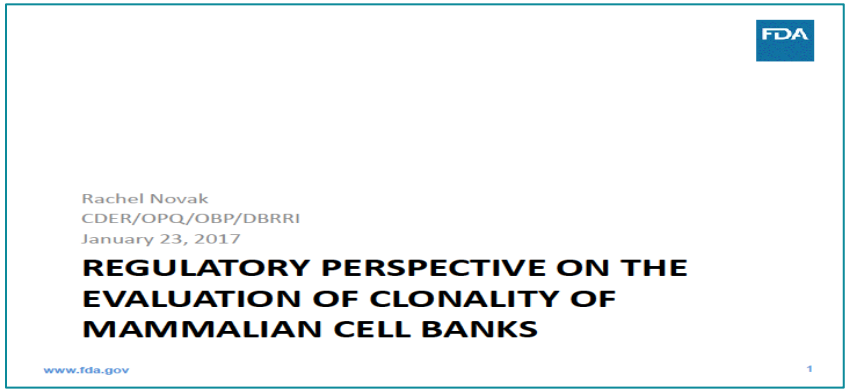
Critical Points

Cell Line Development is not done under GMP; however, a **very high level of documentation** is required

Further, since app 2010, FDA has intensified focus on clonality documentation

The following points are critically important, regardless of whether CLD is done in-house or with a service provider

1. Clonality method and documentation
2. Cell Line Generation report, ideally in a format directly applicable for insertion in regulatory filing document
3. Host cell line history documentation
4. Raw material (BSE/TSE) documentation

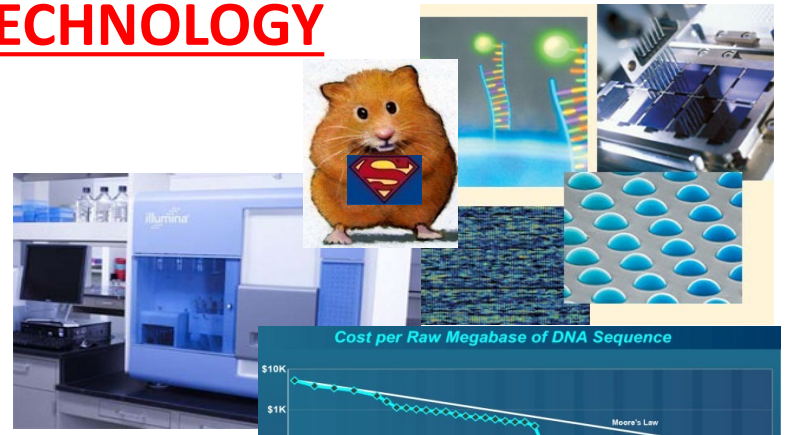


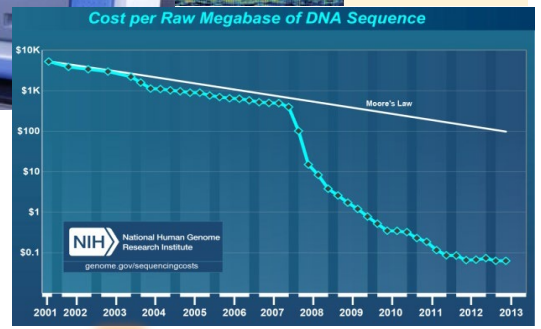
Relevant regulatory guidelines:

- ICH Guideline Q5B Analysis of the expression construct in cells used for production of r-DNA derived protein products
- ICH Guideline Q5A Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.
- Annex 4 Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology
- WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products.

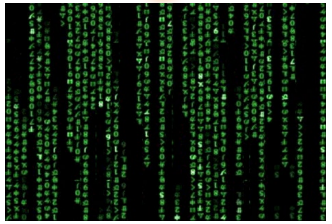
NGS and 'Omics' Technologies has Enabled Detailed Insight in the CHO Cellular Machinery

TECHNOLOGY





Next Generation Sequencing: Can decipher entire genomes or transcriptomes in a few days



Key publications

CHO-K1 draft genome & transcriptome

Xu et al (2011), Nat Biotech 29, 735-741.

***Cricetulus griseus* draft genome & transcriptome & SNP analysis of various CHO lines (K1, S, DG44…)**

Lewis et al (2013) Nat. Biotech

Various RNAome data (miRNA, scRNA, snoRNA …)

E.g. Hackl et al (2011), Lin et al, (2011), Druz et al, (2013), Strotbek et al, (2013)

On top of publications: Biopharma companies make their own studies of their host cells genetic profile, and they dont always publish

Key learning from CHO cell studies of chromosomal make-up:

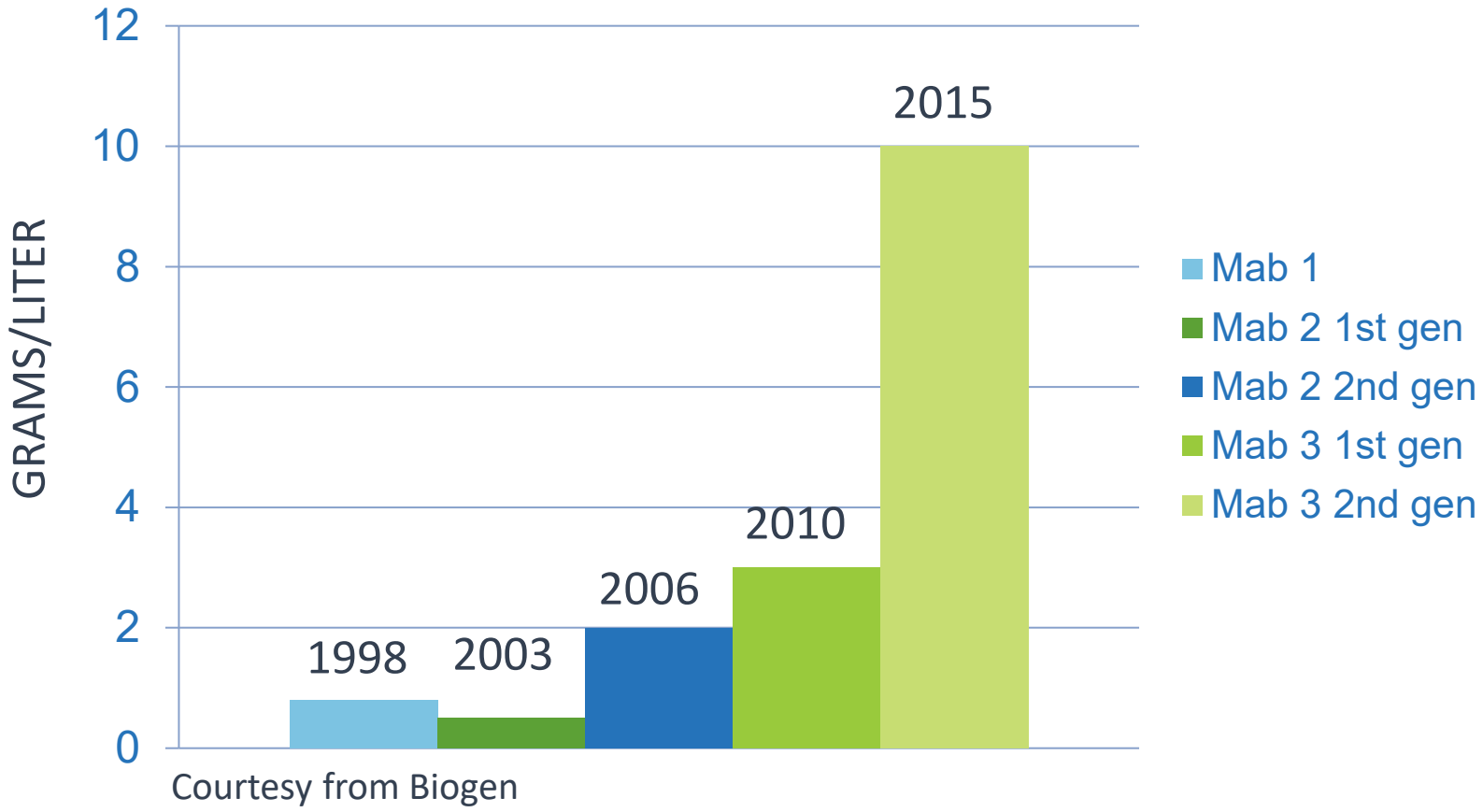
"CHO is so genetically diverse and that explains why you see so many different phenotypes".

Antibody Manufacturing Titer History

- Original titers from CHO cells were in the mg/L range
- Most marketed mAbs were produced with titers < 1 g/L at market entry (and some still are)
- Today‘s scaled-up processes are typically achieving 2-10 g/L
- Latest titer records are reaching 15-20 g/L or more

Commercial Scale Antibody Manufacturing

20 years Productivity Development at Biogen



Courtesy from Biogen

ESACT 2022:
15 g/L titer announced

CHO host cells: More recent Optimization
Examples

- 10x higher ER content for higher productivity and/or faster growth
  - CHO Plus
- Shorter dobling time for faster processes
  - CHO-MK
- Improved efficacy for ADCC MoA, potentially lower dose/cost
  - CHO GlymaxX

Transposase Technology Increasingly used for CLD

About two decades ago, multiple academic laboratories started to explore transposases, their mode of action and their potential for gene transfer.

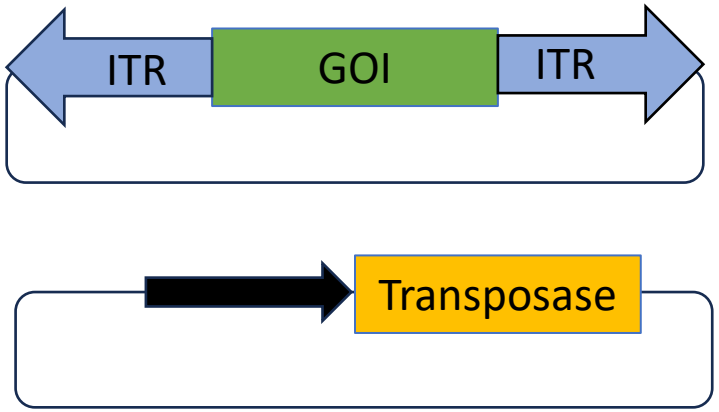
- **Sleeping Beauty** was the first transposon used

The transposases were initially considered risky tools due to potential ”gene jumping”.

However, the technology was seen as advantageous compared to random integration with respect to productivity and stability

Intense research of the technology let to optimizations and generation of artificial transposons designed to mitigate risks

The functional components ie the inverted repeats (ITRs) flanking the GOI and the transposase were split up in two functional components – or the transposon was delivered as degradable mRNA.



Success:

Biopharma/biotech companies and CDMOs have now adapted transposon technology

- BI, Eli Lilly, Lonza, ATUM, ProBioGen are some examples
- Transposons include for example PiggyBac, Leap-In, DirectedLuck

Site-specific Integration Return

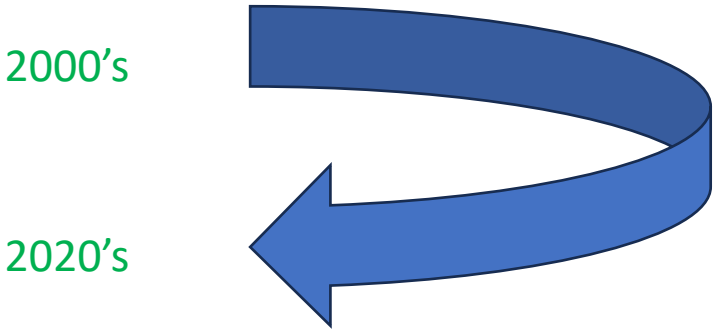
Site-specific integration anticipated advantage:

- Transfer of Gene-of-Interest (GOI) into one **stable** and **transcriptionally active** locus

Examples:

- Flp-In; Cre-Lox; RMCE; attP/attR (lambda integrase); Crispr, TALEN, ZFN

Recent revival driven by: Production of polyclonals/mixtures; Covid need-for-speed (no cloning)



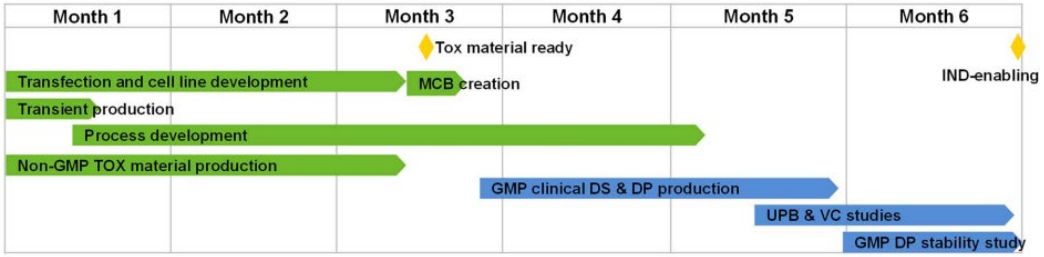
Accelerating Timelines and Streamlining Development Requirements:

How anti-Covid-19 Mab development forced companies and regulatory agencies to rethink

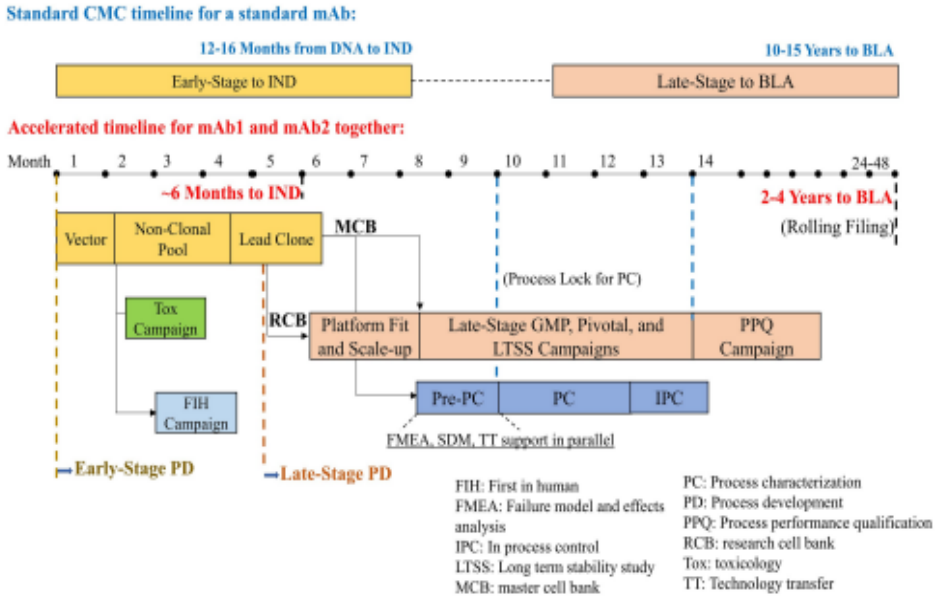
In early 2020, WuXi Biologics mobilized a huge group of scientists (>200) and worked with multiple clients across the globe to accelerate the development of potential antibody-based COVID-19 treatments.

Tan KW et al, (2024): Further accelerating biologics development from DNA to IND: the journey from COVID-19 to non-COVID-19 progr
Antibody Therapeutics 2024

Antibody Therapeutics, 2024



Bristol-Myers Scuibb has published a similar story comparing the accelerated setup to their standard CMC approach.
Xu-J, Ou-J, McHugh-KP, Borys-MC, Khetan-A (2022): MAbs, vol 14 no 1, e2060724



Many other biotech and biopharma companies have used similar strategies: Regeneron, Catalent, AstraZeneca, Lilly, GSK…

Regulatory agencies have been involved concurrently and have approved the novel concepts.

It will be very interesting to see how these approaches may influence CMC development guidelines in the future.

Covid-19 mAbs paved the way for accelerating timelines to the clinic

Pre-clinical development timelines were reduced from app 1-1,5 year to 0,5 year

Key instruments used to achieve this:

- IND enabling CMC/emergency use/conditional marketing authorization:
  - **Stable pool production**; **highly integrated workflows (CMC/clinical); risk taking; close interaction between industry and regulatory authorities**
- Late-stage and commercial stage CMC viable strategy based on:
  - **In-process controls; comparability; facilitated post-approval changes**

There is time, but is there a need to exchange the IND-enabling production cell with a ”clone” from a scientific – or cost-of-goods - point of view?

Is there a sufficient data-basis now to update existing ICH and regional guidelines?

Recent CLD Key Publications on Speed-to-Clinic and Clonality

Kelley B (2020) Developing therapeutic monoclonal antibodies at pandemic pace. Nature Biotech 2020 (**27 companies**)

Zheng Zhang et al (2021): Reshaping cell line development and CMC strategy for fast responses to pandemic outbreak. Biotech Prog 2021

Agostinetto R et al (2021): Rapid cGMP manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools. Biotech Bioeng 2021

Xu G et al (2022): Quality comparability assessment of a SARS-CoV-2-neutralizing antibody across transient, mini-pool-derived and signle-clone CHO cells. Mabs 2022

Xu J et al (2022): Upstream cell culture process characterizatoin and in-process control strategy development at pandemic speed. Mabs 2022

Broly H et al (2023): Effects of the COVID-19 pandemic: new approaches for accelerated delivery of gene to first-in-human CMC data for recombinant proteins. Mabs 2023

Wang et al (2023) Accelerating the speed of innovative anti-tumor drugs to first-in-human trials incorporating key de-risk strategies. Mabs (2023)

Higgins MF et al (2023): Accelerated CMC workflows to enable speed to clinic in the COVID-19 era: A multi-company view from the biopharmaceutical industry. Biotechnol Prog 2023 (**8 companies**)

Tan KW et al, (2024): Further accelerating biologics development from DNA to IND: the journey from COVID-19 to non-COVID-19 programs. Antibody Therapeutics 2024

Clarke et al (2024): When will we have a clone? An industry perspective on the typical CLD timeline. Biotechnol Prog. (**10 companies**)

Not a complete list

EFPIA, BioPhorum …. Collaborative efforts behind much of this work

Conclusions

- Immense improvements of expression stability, faster timelines and much higher titers have been gained since the first attempts to produce biologics in mammalian cells
- The CHO genome availability was key to the wave of engineering approaches seen over the past decade
- Recent technological highlights include use of transposons and the come-back of site-specific integration
- COVID induced a wave of fast-time-line approaches – interesting to see how this will influence future approaches
- Optimization of CLD elements always go hand-in-hand with process and media optimization and integration

Together, these aspects have made a huge impact on development timelines
from candidate selection to the patient

Frederiksborg slot/Hillerød Castle, Denmark