AbtBioConsult

Supporting biologics CMC development

ACT in a Nutshell

Industrial Manufacturing of Therapeutic Proteins using ACT

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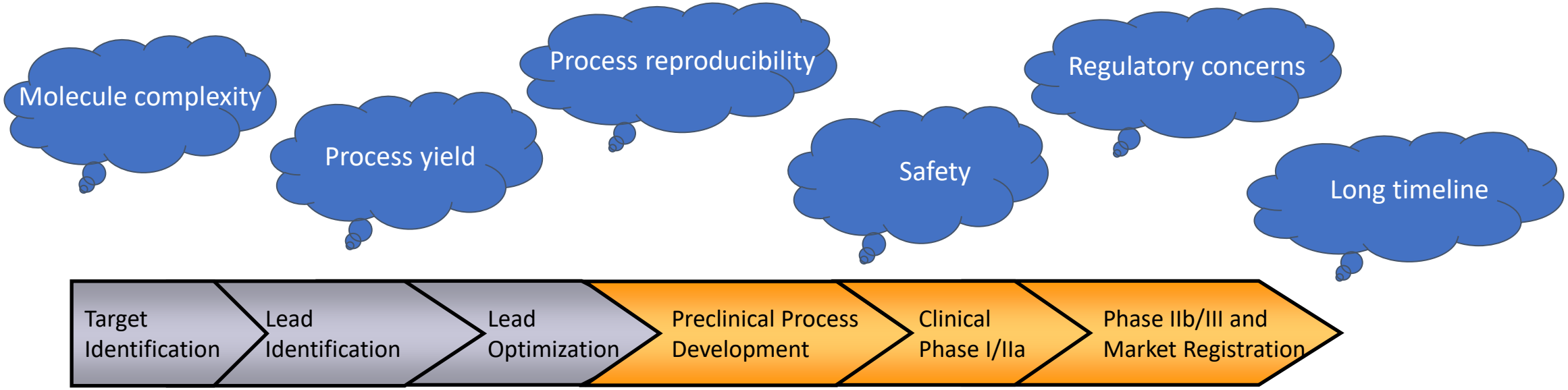
How can we ensure fast development of mammalian manufacturing processes for production of biological human therapeutics which are robust, high-yielding and with the desired product quality?

1. Regulatory Requirements
2. Chinese Hamster Ovary cells – history and use
3. Improved Manufacturing Process Technologies
4. More Efficient Drugs
5. Biopharmaceuticals Industry Trends

Regulatory Requirements

From Mind to Market: Biopharmaceutical Drug Development

Biopharmaceuticals CMC development is very expensive. Why?



However, these timelines are changing - due to the Covid pandemic

The DNA sequence of the Covid virus (SARS-CoV) was published in **January 2020**.

Already in **November 2020**, the first two anti-SARS-CoV antibody products were authorized for emergency use by FDA (one mAb, one Ab cocktail).

Good Manufacturing Practise (GMP) for Human Therapy Biologics Production

Manufacturing Requirements. A few examples:

- GMP Manufacturing Cell Lines need to follow multiple regulatory guidelines, covering for example safety testing for identity, sterility and adventitious viruses and genetic characterization (ICH Q5A, ICH Q5B, ICH Q6B, ….)
- Mammalian purification processes must contain **two orthogonal virus** removal steps
- Virus validation studies required for each purification process
  - Scale-down model establishment
  - Spiking experiments with model viruses
- Qualification/validation of all equipment required
- Qualification/validation of analytical methods required
- Release test of all raw materials required

GMP Manufacturing Requirements: A few more Examples

Generate and maintain **Batch Manufacturing Record** of all operational steps during production

Performer/verifier (2 persons) required for all critical operations

Classified manufacturing rooms

Environmental monitoring (EM) for microbials

Release testing of ALL batches – typically 15-20 analytical methods plus functional assay

This is hard work!

Clinical Trials – Making Sure the Drug is Safe and Efficient

Phase I:

Initial safety trial on a new medicine to establish the dose range tolerated by about 20-30 healthy volunteers. Sometimes conducted with severely ill patients, for example those with aggresive cancers.

Phase IIa/b:

Clinical trials to evaluate safety and efficacy in relevant patients. Typically 100-300 patients.

Phase III:

Multicenter studies on 1000-3000 patients (or more). Phase III trials generate additional safety and efficacy data from relatively large numbers of patients in both controlled and uncontrolled designs and are used to support a BLA (Biologics License Application)/MAA (Market Autorization Application).

Market Entry after ~10-15 years

And there is more: Phase 4 post-launch studies also required

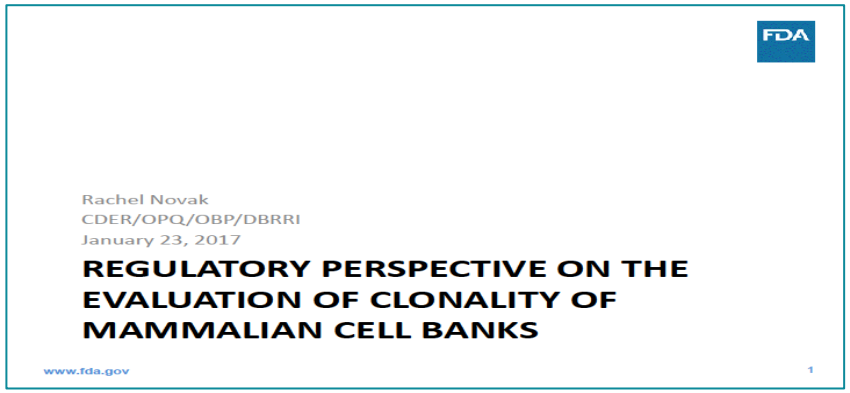
CLD Documentation Requirements
Critical Points

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

Further, since app 2008, FDA (Sarah Kennett) 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.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Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Industry view on the relative importance of "clonality" of biopharmaceutical-producing cell lines

Christopher Frye [a, *], Rohini Deshpande [b], Scott Estes [c], Kathy Francissen [d], John Joly [d], Anthony Lubiniecki [e], Trent Munro [b], Reb Russell [f], Tongtong Wang [a], Karin Anderson [b]

ARTICLE INFO

ABSTRACT

1. Introduction

1.1. Historical perspective

Clonality!

Production clones 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

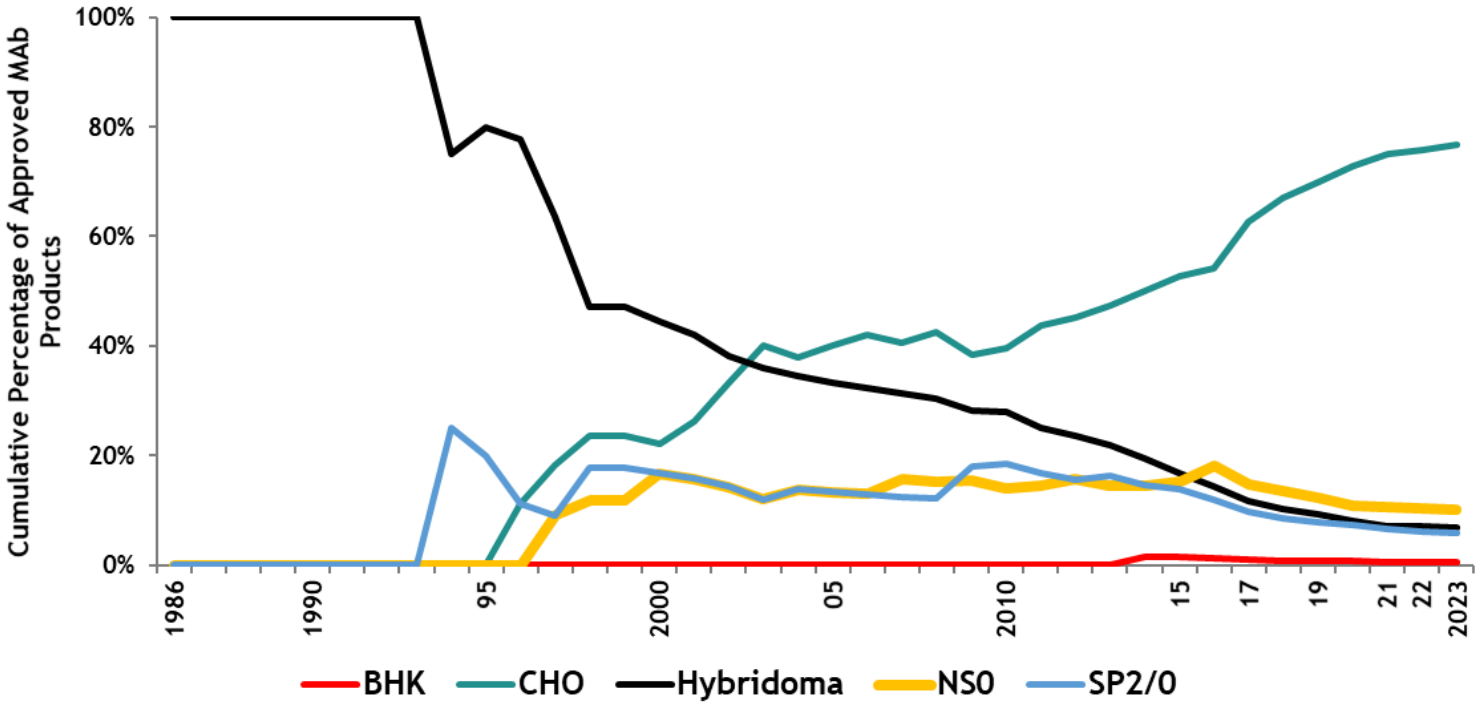
Cell printer + imaging

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

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

Chinese Hamster Ovary cells – history and use

Host Cells for the Production of Antibody Products



- In 2023, CHO cells comprised 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

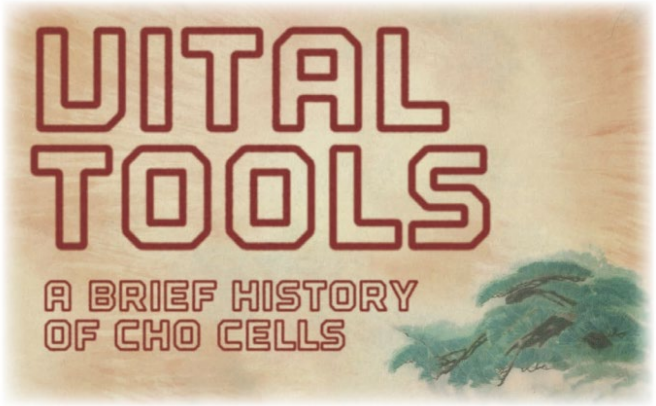
- **Chinese Hamsters** were imported to North America from China during the Chinese civile war in **1948**
- Dr. Theodore Puck established the first CHO cell line in **1957**
- Since 1968, **adherent** CHO cells are available for purchase, for example from ATCC (CHO-K1)
- Since 1980, Dr Lawrence Chasin, 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 can be purchased (CHO-S)

Note:

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

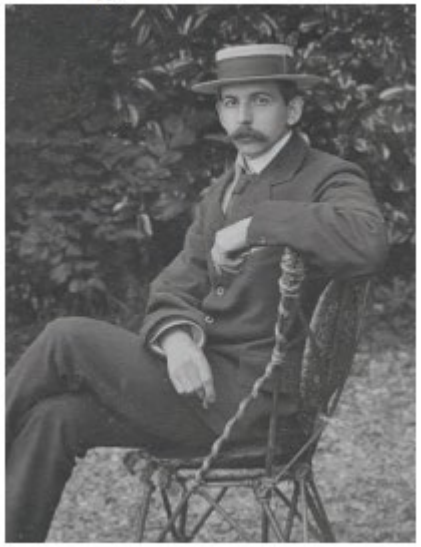
Chinese Hamsters travelled from China to North America during the Chinese Civil War in 1948

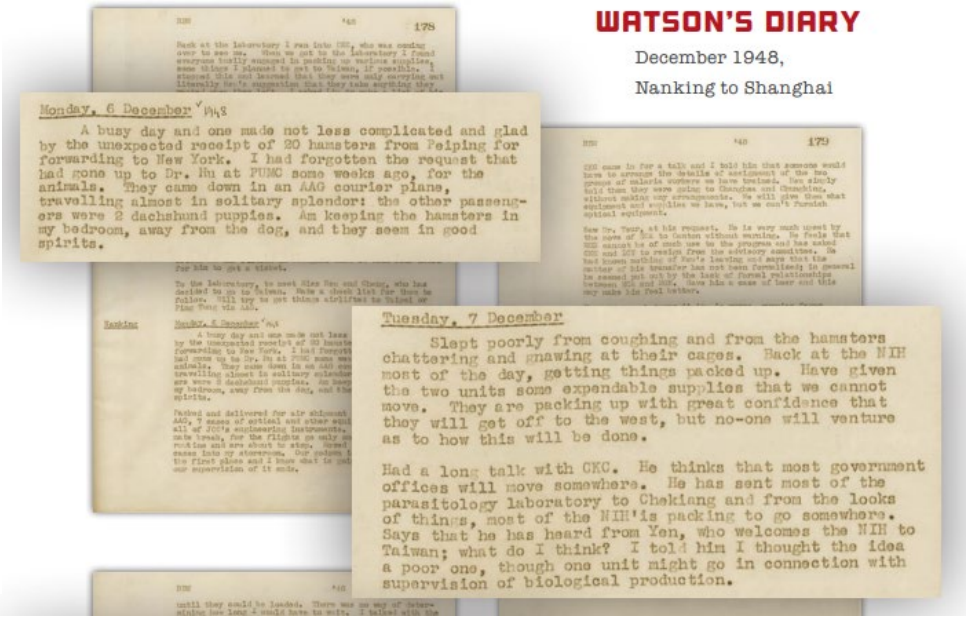
They barely made one of the last Pan-Am flights out of Shanghai before the Maoists claimed victory



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

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Know all components of your technology platform

Example: CHO Cells are not Just CHO cells

Hamster → CHO (Isolated by Tijo & Puck 1958) → CHO Pro- (Proline-dependent mutant, Puck et al. 1967)

subclones of Pro-

- **CHO-S** (Invitrogen) → **CHOK1SV** (Lonza)
- **CHO-K1** (Puck et al., 1968, ATCC CCL-61) → **DG44** (Urlaub & Chasin 1980) → **HEX1**, **HEX2** (Boehringer-Ingelheim), **ECHO** (Symphogen)
- **DuxB11** → **DP12** (Genentech)
- **Pro-5** (P. Stanley, Cell 1975, ATCC CRL-1781) → P. Stanley → **Lec mutants**

Wild type

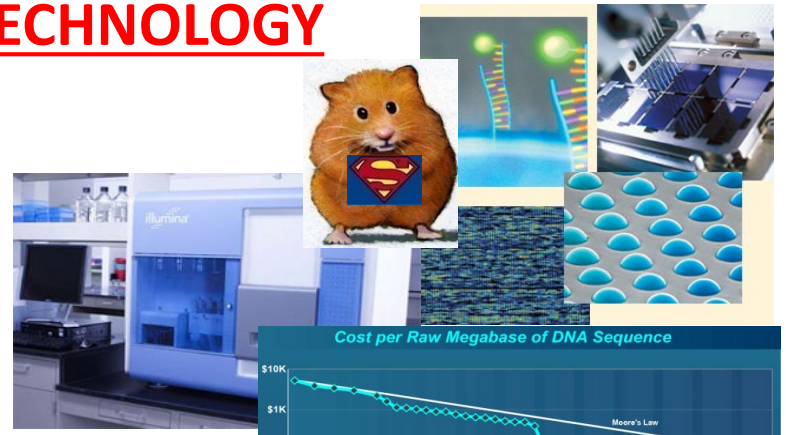
dhfr -/-

Not for use/distribution

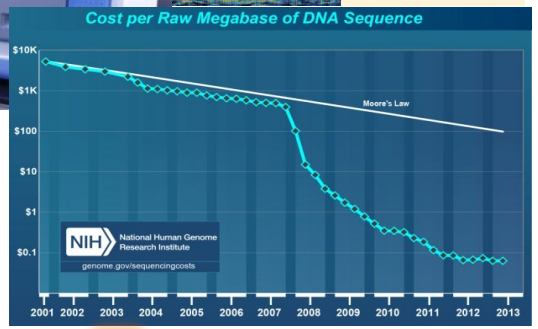
Most BioPharma and Contract Manufacturers developing biopharmaceuticals have their own CHO host cell as an integral part of their platform

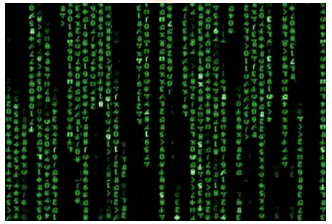
NGS and 'Omics' Technologies has Enabled Detailed Insight in the CHO Cellular Machinery

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 cells are so genetically diverse which explains why you see so many different phenotypes".

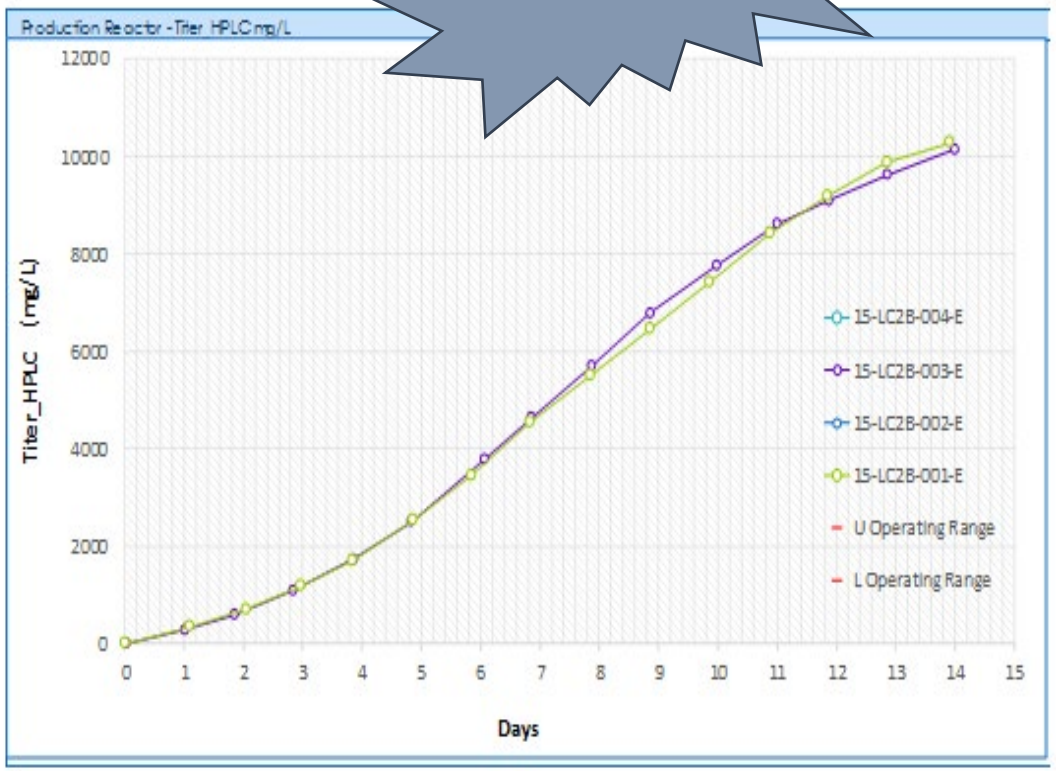
Antibody Manufacturing Upstream Process Success

- Original titers from CHO cells were in the mg/L range (1980-1990ies)
- Most marketed mAbs were produced with titers < 1 g/L at market entry
  - - and some legacy products still are today
- Today‘s scaled-up processes are typically achieving 2-10 g/L
  - - highly recombinant molecule dependent
- Latest titer records are reaching 15-20 g/L or more

20000 L Production Results

- ✓ Seeding 20 kL bioreactor with 10 million cells/ml after N-1 perfusion stage
- ✓ Successful demonstration of automated process control via new technologies
- ✓ Feasibility of harvesting supernatant from a high cell density and high titer process confirmed
- ✓ Production of **> 170 kg** antibody in a single batch!

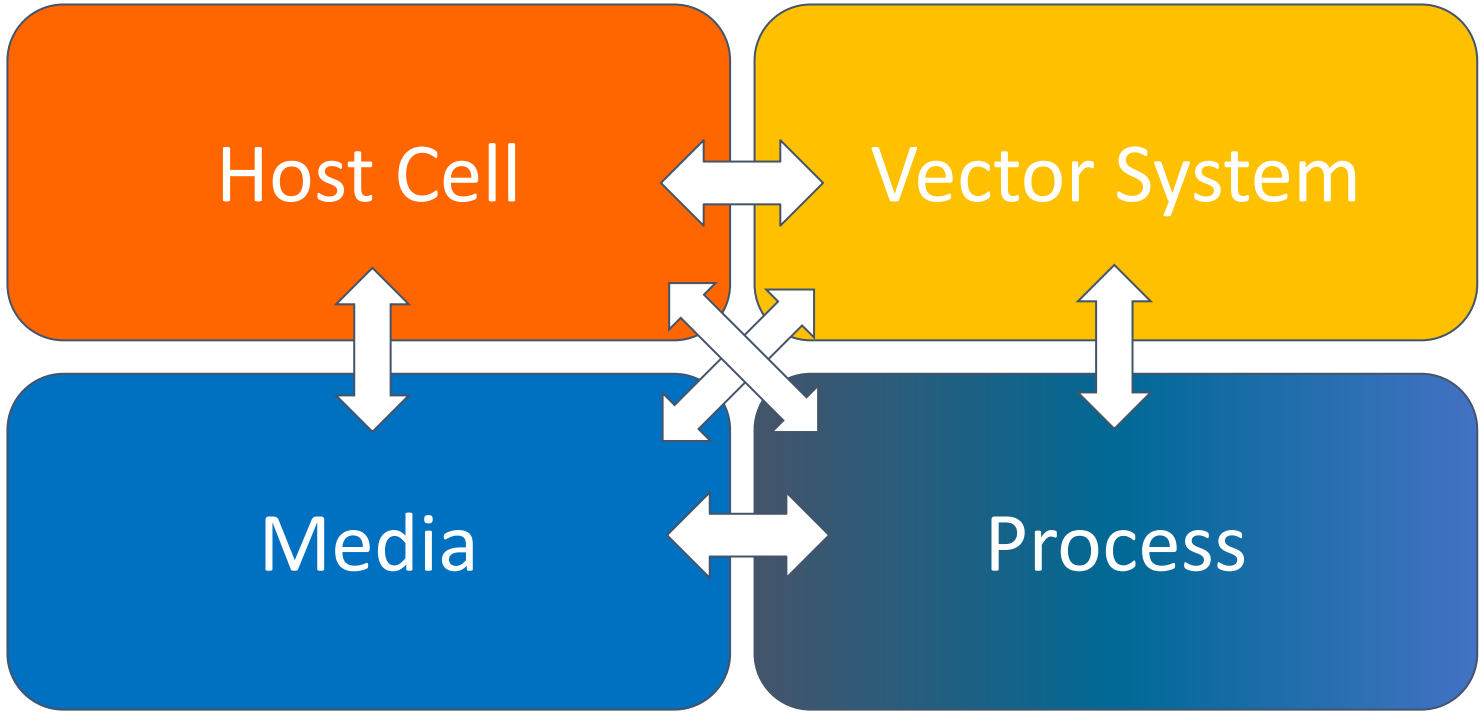
10.2 g/L at 20kL scale!



Courtesy from Biogen

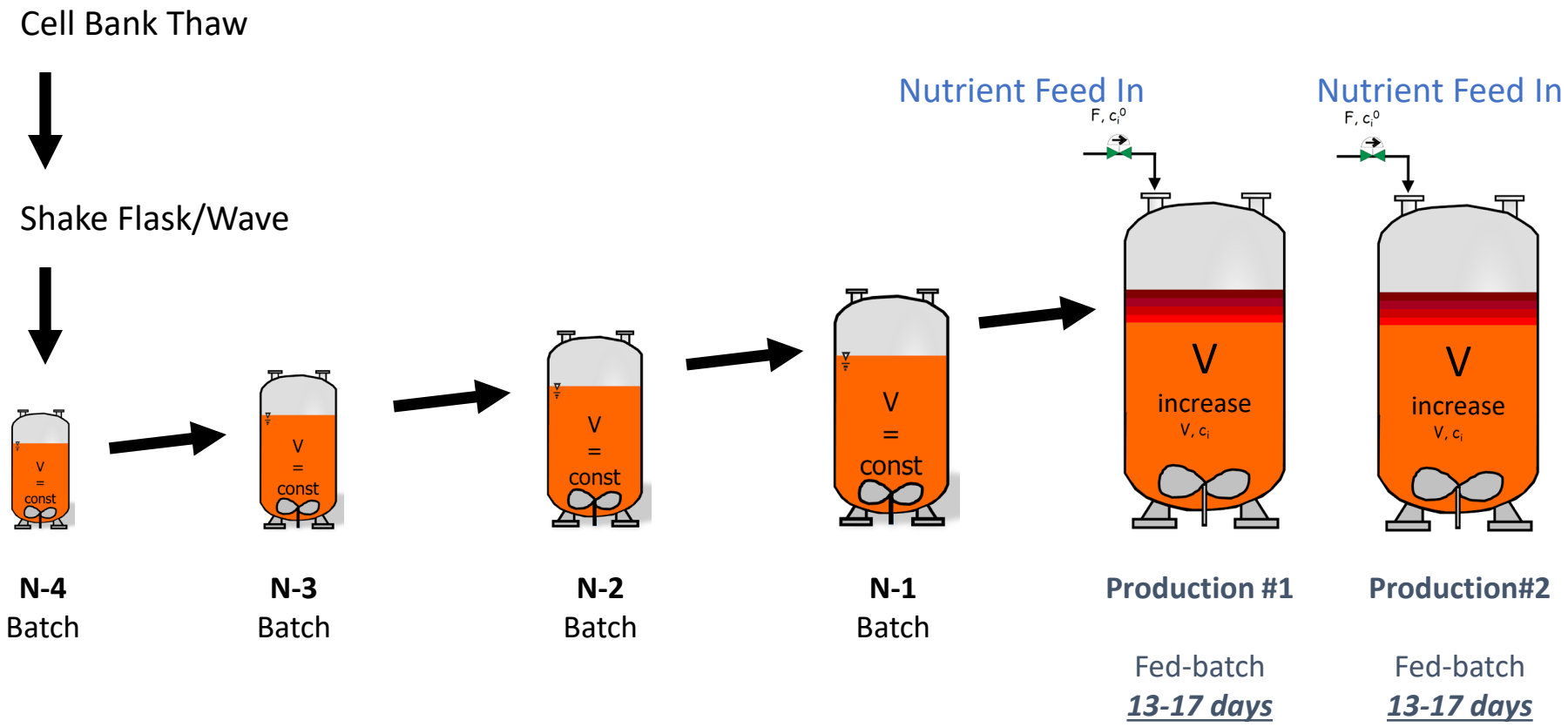
Improved Manufacturing Process Technology

The Four Building Blocks of an Upstream 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

A Typical Fed-batch Cell Culture Process



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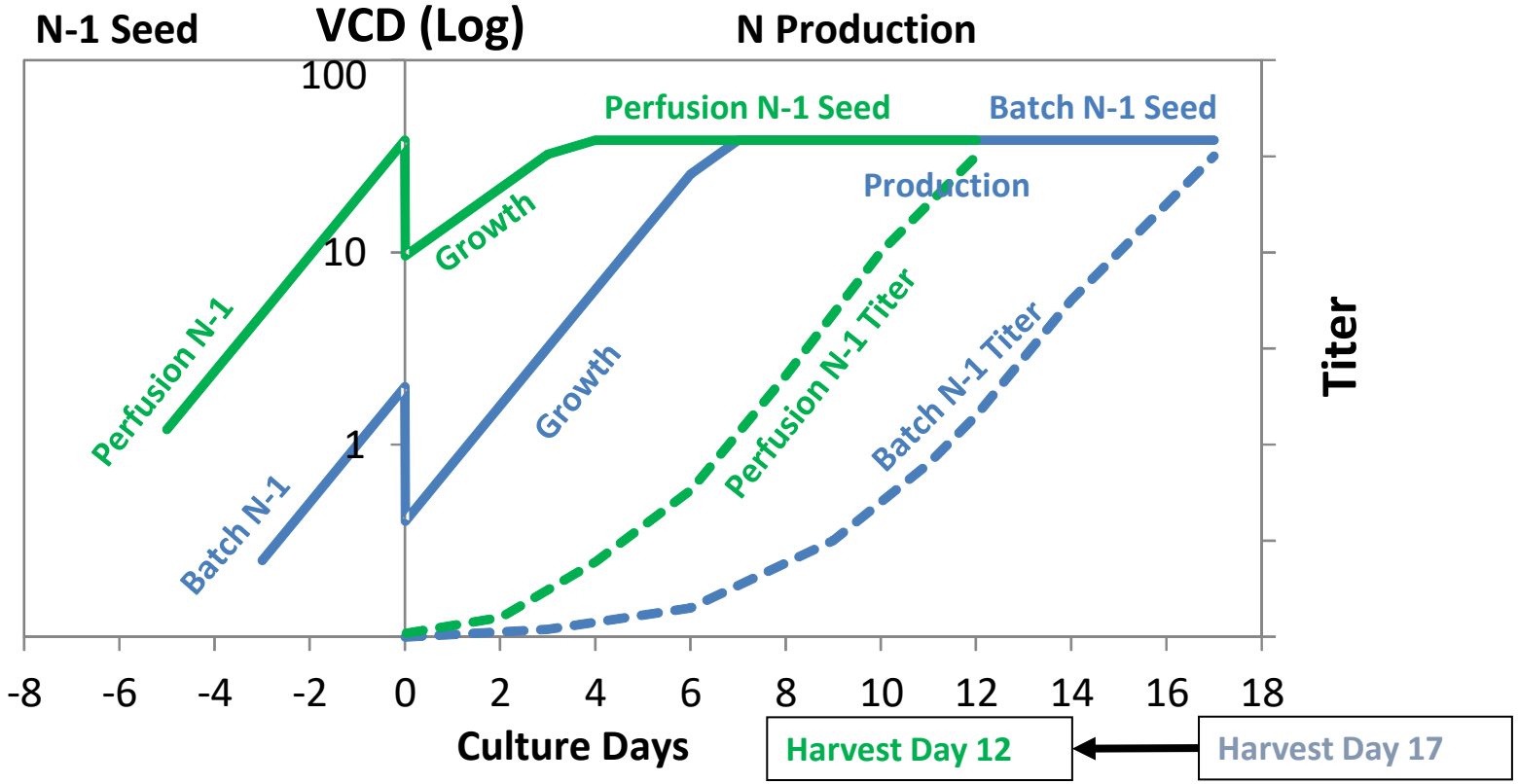
Perfusion at N-1 Shortens the Production (N) Stage



- **Shift growth phase to N-1 stage**
  - Very high-seed production cultures to **shorten culture duration**
  - **More batches** in the same amount of time
  - Increase production capacity by more efficiently utilizing the ratio between N-1/N stage bioreactors

Courtesy from Biogen

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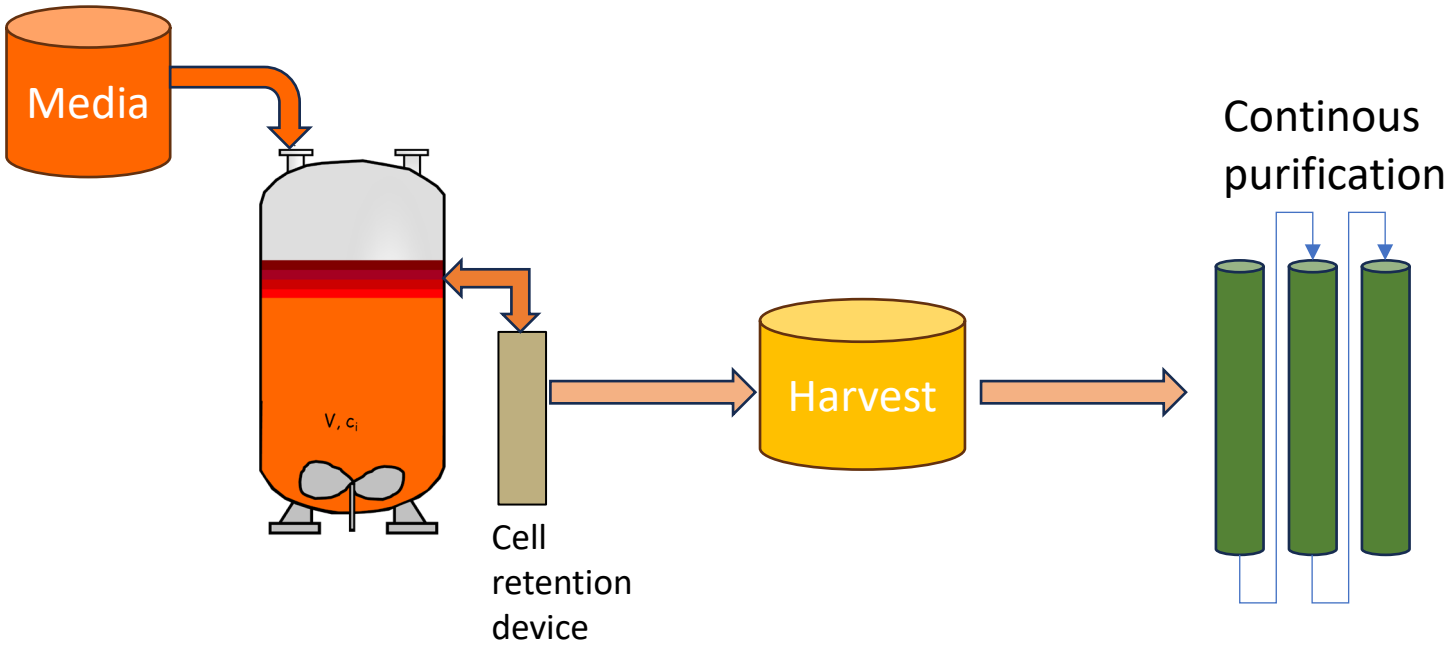
Perfusion and Continous Processing is a new – and old - trend

- Operation modes
  - Production tank perfusion
  - N-1 stage perfusion
  - Some kind of hybrid process
  - Current trend: Continous downstream processing



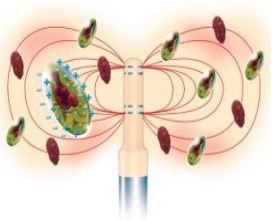
- Some advantages
  - Smaller bioreactors = smaller facility footprint
  - Lower construction costs
  - Higher purity
  - Better cell viability and (maybe) product quality

- Some challenges
  - Longer processes (typically)
  - Higher complexity
  - Quality differences during manufacturing
  - Higher contamination risk
  - Longer process development

Measuring, Monitoring, and Control of Bioprocesses - **PAT**

The American Food and Drug Administration's (FDA) guidance on **Process Analytical Technology** (**PAT**) came out in 2004

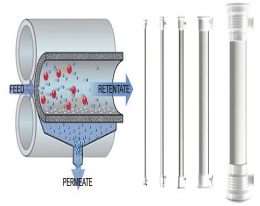
Biocapacitance-based nutrient feeding



Raman-based glucose feeding

Hollow fiber perfusion filters

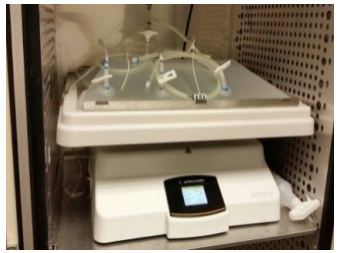
N-1

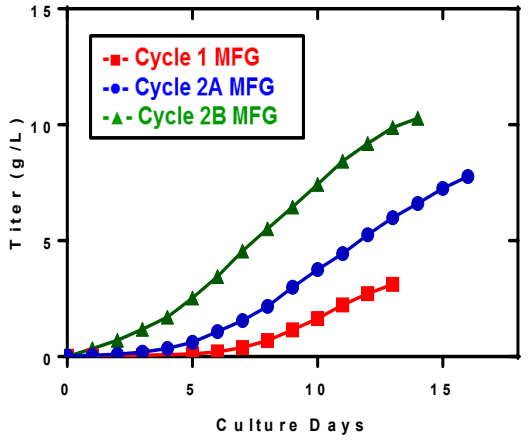


15000 L scale

High density working cell bank

Wave technology





PAT methods for advanced process control along with modern equipment assure process and product quality

Courtesy from Biogen

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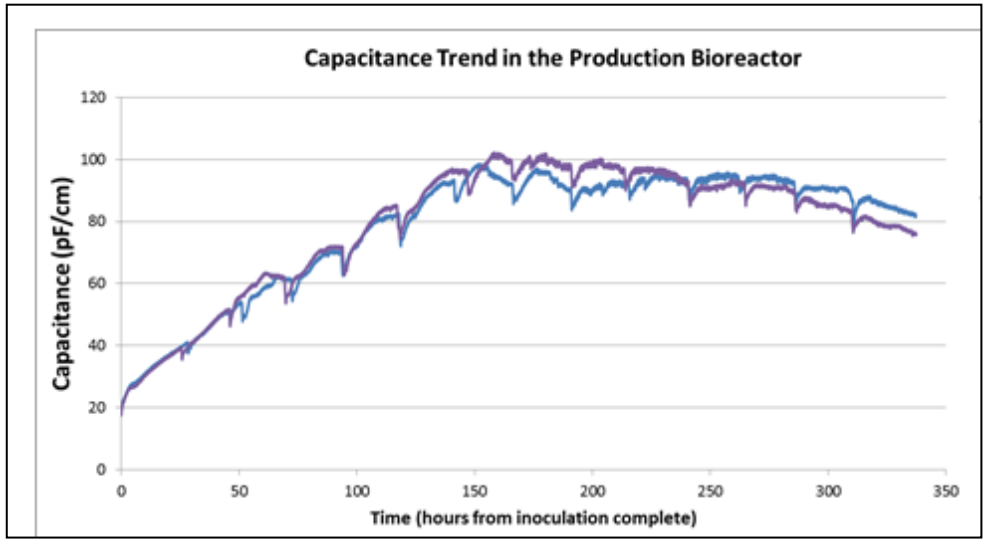
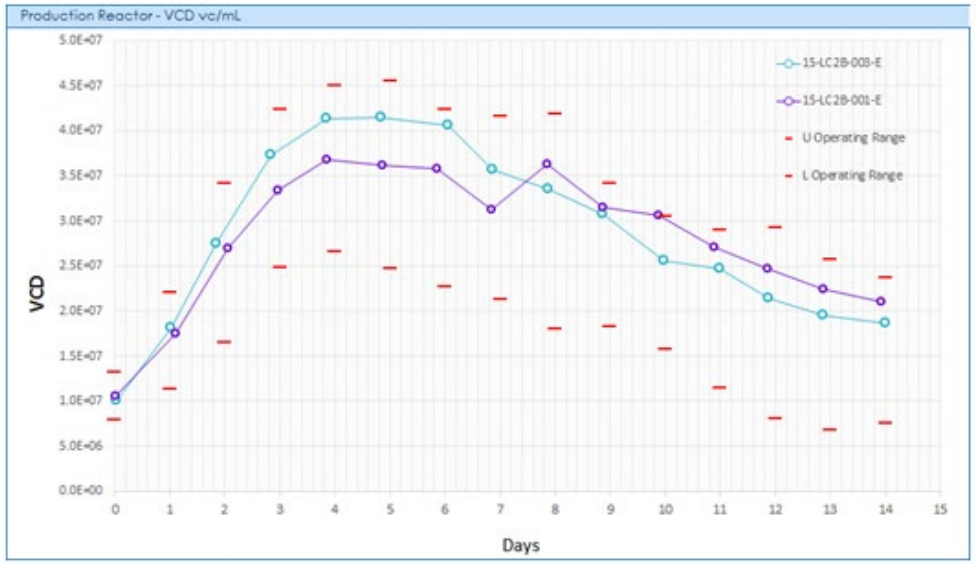
Biocapacitance vs off-line Cell Counting





- On-line monitoring of cell growth generated continous data-points
- Enables operators to follow the batch performance very closely
- Off-line monitoring of cell growth generating discrete data points – typically one per day(!)
- Less chances to detect unexpected growth performance

More Efficient Drugs

Nobel Prize Winning Science 2018

Immuno-Oncology (IO) Treatment Concept using Checkpoint Inhibitors

- Tasuku Honjo and colleagues first described the programmed cell death 1 (PD-1) protein in 1992
- In 1996 James Allison and colleagues reported that blocking CTLA-4's inhibitory effects improved immune responses directed toward tumor cells.

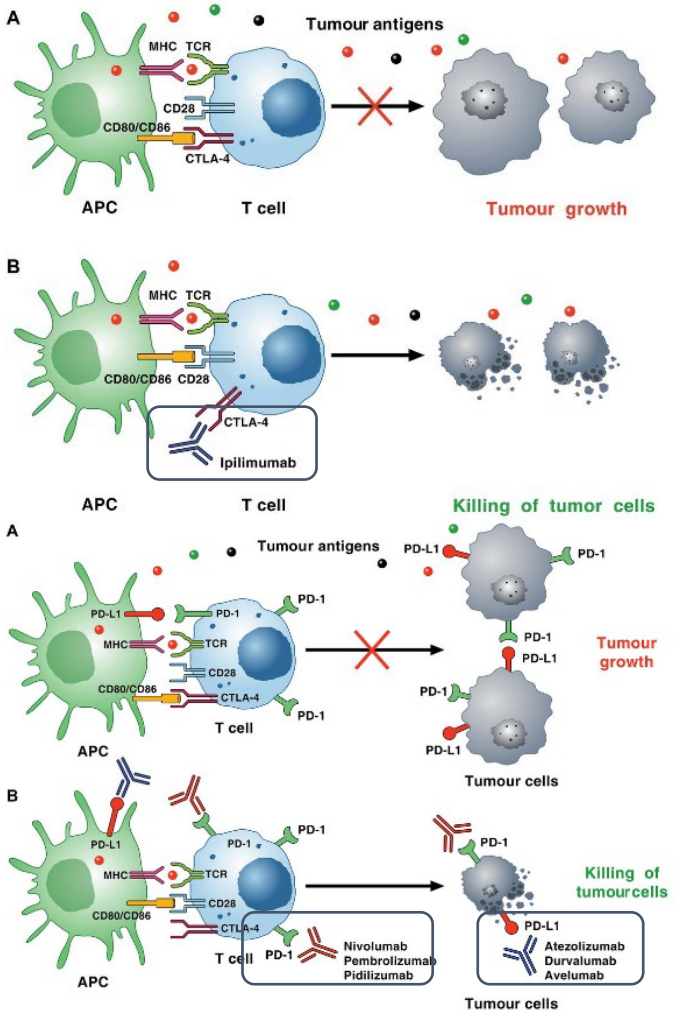
Checkpoint inhibitors

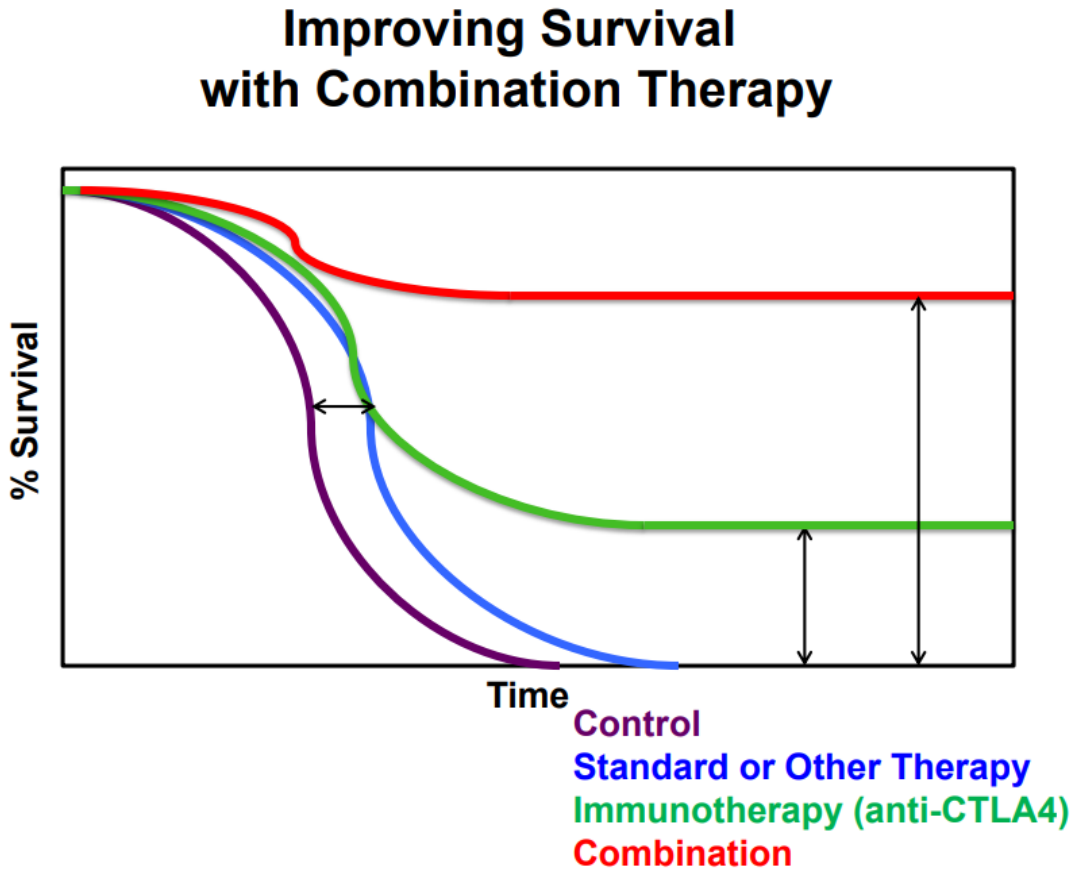
Antibody drugs developed based on these discoveries:

- **Ipilimumab (Yervoy®):** a checkpoint inhibitor that targets the CTLA-4 pathway; approved for subsets of patients with advanced melanoma, including as a first-line therapy
- **Nivolumab (Opdivo®), Pembrolizumab (Keytruda) and several others:** checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with advanced melanoma

Dramatic Increase in Cancer Survival (Malignant Melanoma)

Blocking CTLA-4 and PD-1 with antibody therapeutics





From Jim Allisons Nobel Prize Lecture, Dec 2018

Varrichi-G et al: ESMO Open. 2017 Oct 26;2(4):e000247. doi: 10.1136

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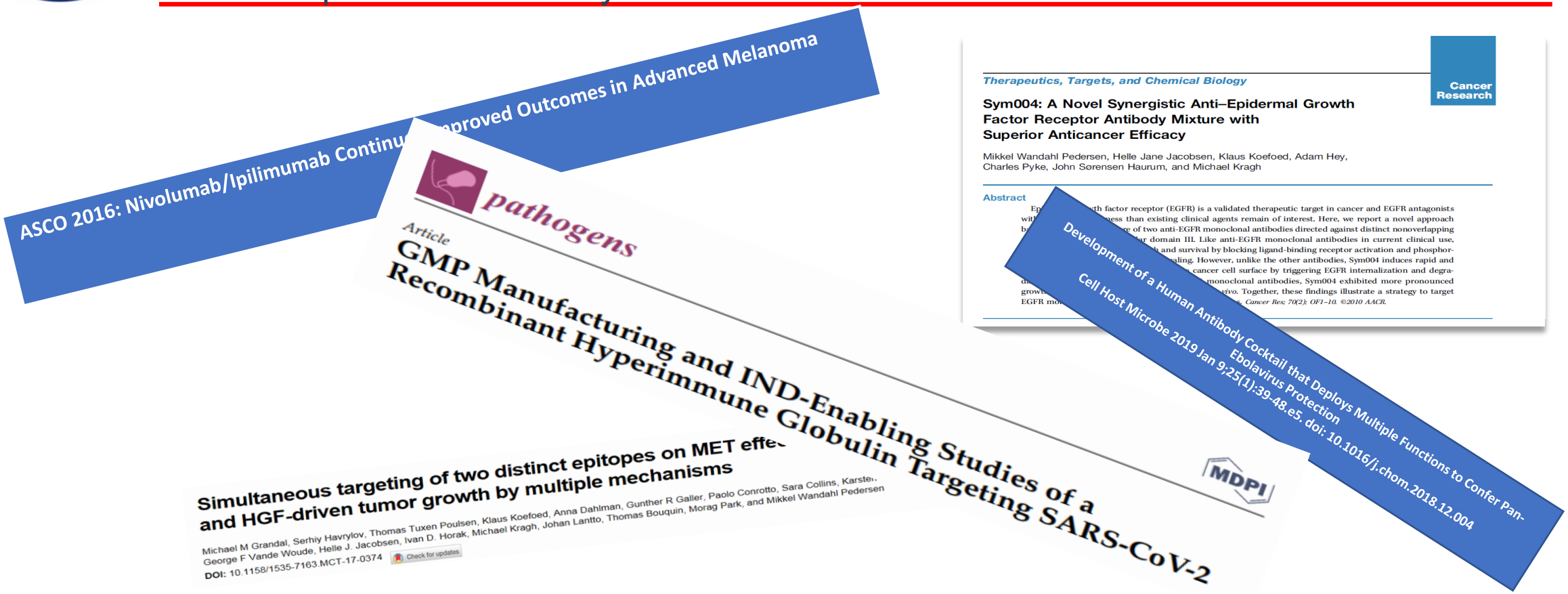
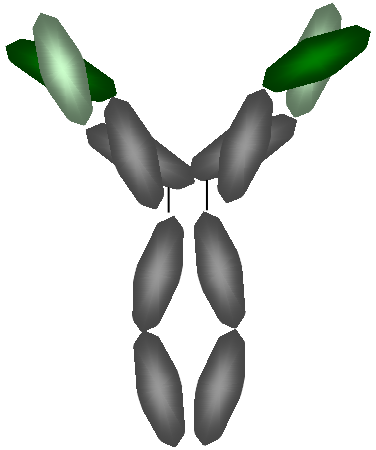
Some (maybe most) Diseases are not Cured by Targeting a Single Epitope: Trend for Development of Antibody Mixtures



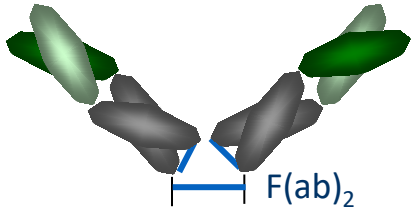
Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies
Science 2020 Aug 21;369(6506):1014-1018.
doi: 10.1126/science.abd0831. Epub 2020 Jun 15.

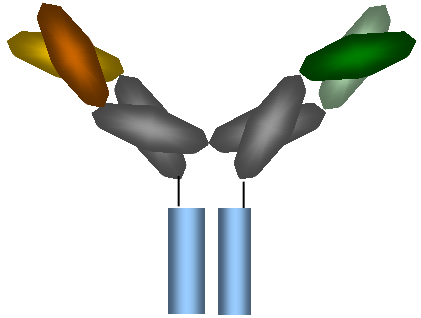
Antibody Derivatives including bi- or multispecifics and ADC-conjugates are trending

Monoclonal Antibody
(IgG)



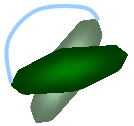
Fab Fragments



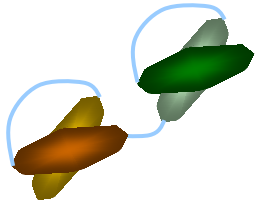


F(ab)$_2$ (bispecific)

Single chain fragments

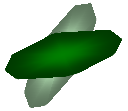


scFv



Bis-scFv (bispecific)

Single domain fragments

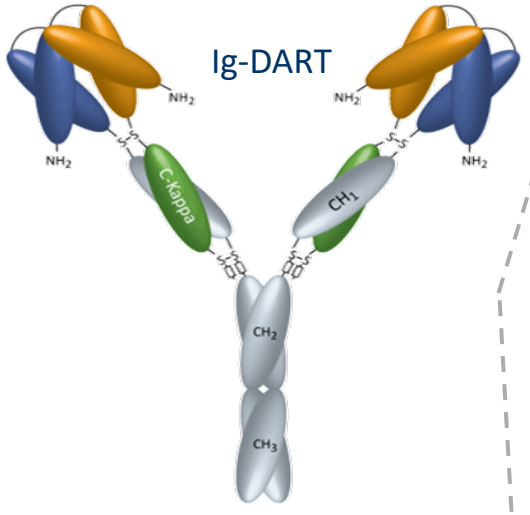


Fv

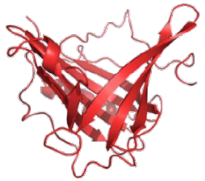
Higher efficacy

V_{HH}

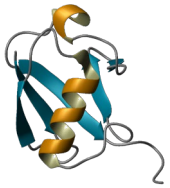
Nanobodies®



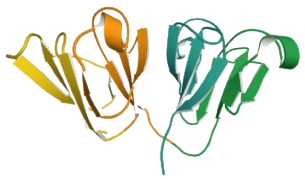
Anticalin



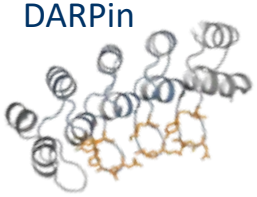
Ubiquitin



Crystallin

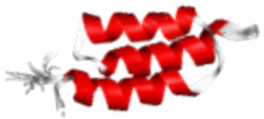


DARPin



Aptamer

Affibodies



Tetranectins

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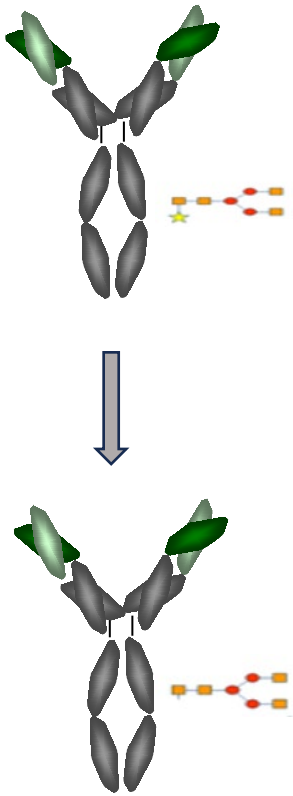
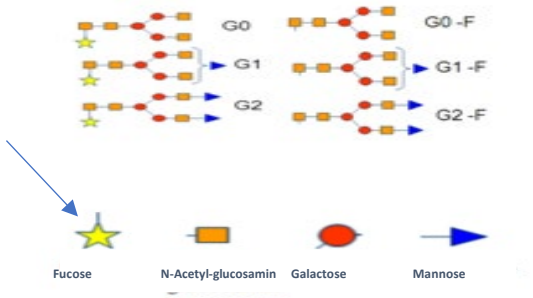
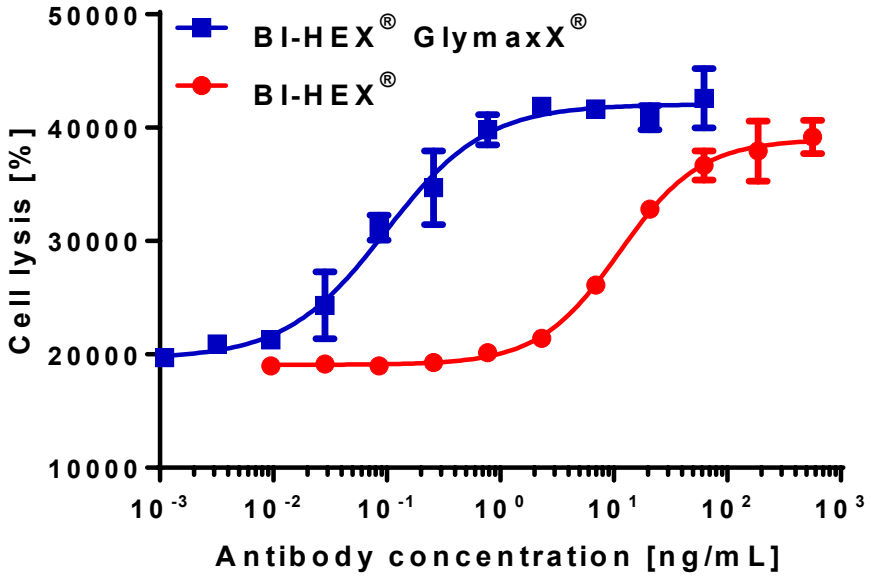
Modified Host Cell Line for Manufacturing of mAbs with high ADCC Activity

Example BI-HEX® GlymaxX®:





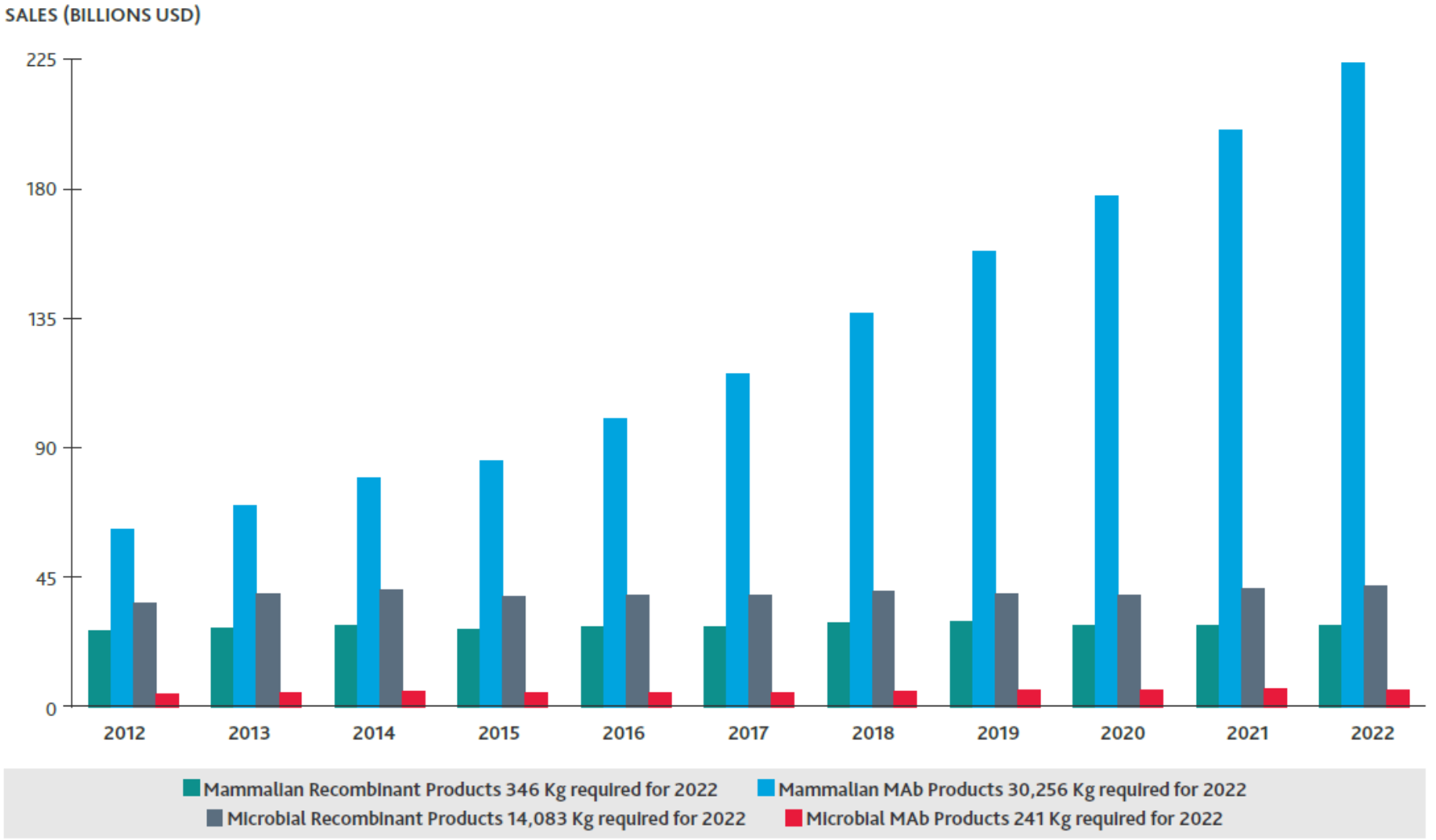
ADCC activity result



BI-HEX®-GlymaxX® produced mAbs are 20 -100 fold more active in ADCC assays
BI-HEX®-GlymaxX® produces >90 % defucosylated antibodies

Biopharmaceuticals Industry Trends

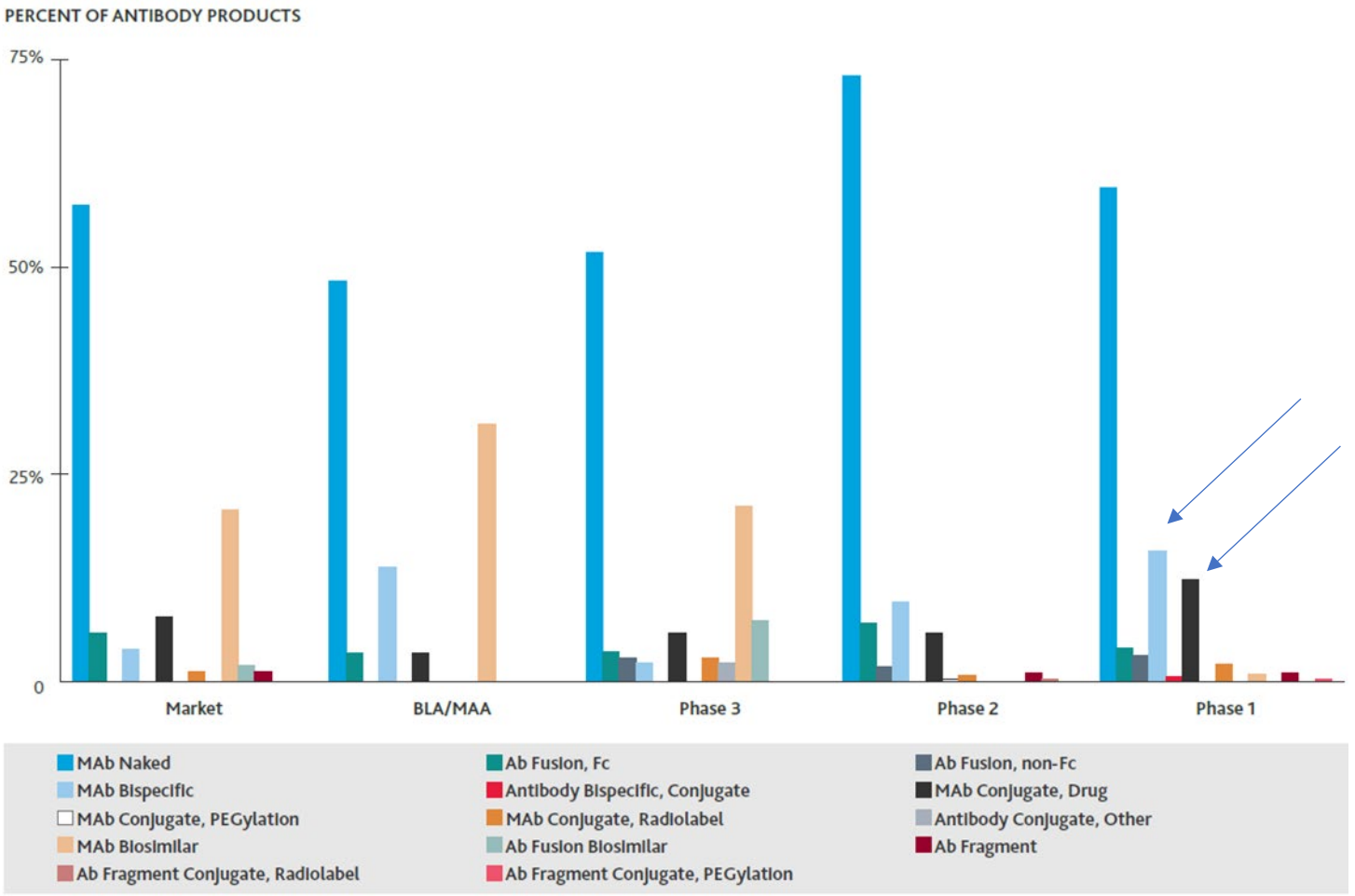
Growth and Distribution of the Biopharmaceutical Market



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

Not for use/distribution

Mammalian Biologics Product Distribution by Product Type and Phase



- 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%
- Bispecifics constitute the following proportions of the MAb pipeline:
  - Marketed Products: 4%
  - BLA/MAA: 14%
  - Phase 3: 2%
  - Phase 2: 10%
  - Phase 1: 17%

Data and graphs derived from BDO's BPTG bioTRAK® database:
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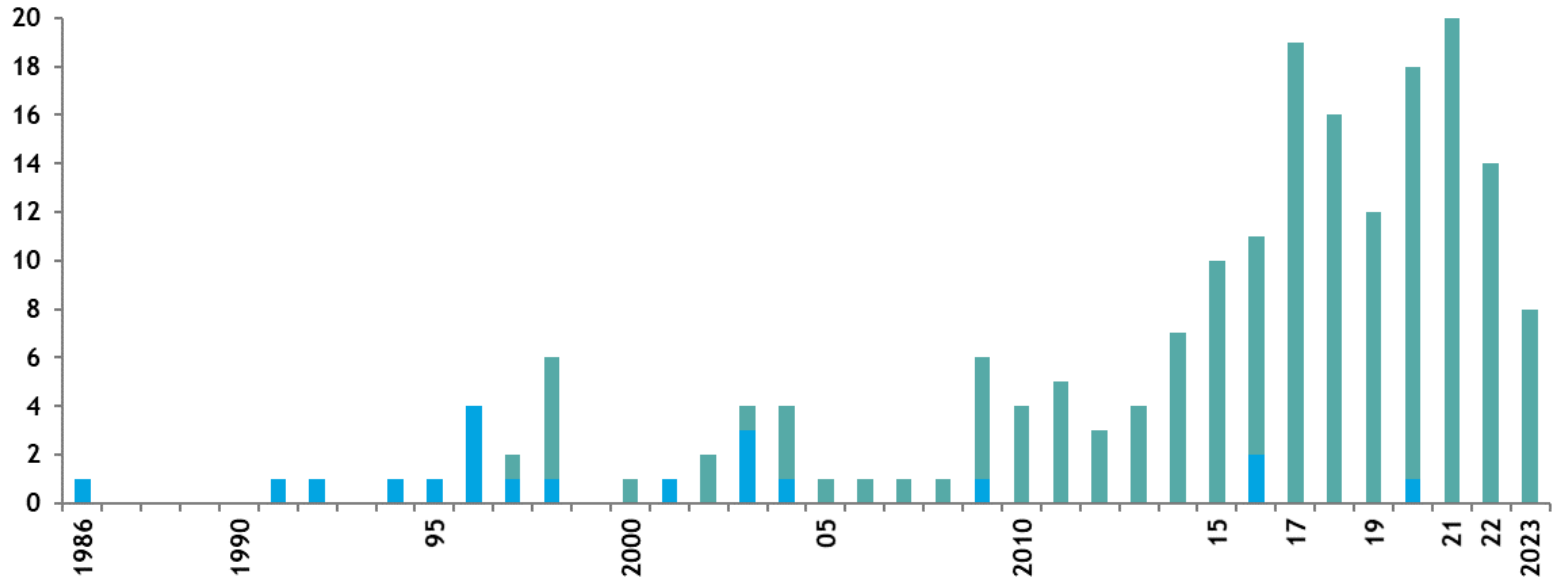
Antibody Products Approved for Human Therapeutic Use since 1986



- Products approved but subsequently removed from market

- Antibody Products include: MAbs, bispecific MAbs, Antibody Fragments, Antibody Fusion Proteins, Biosimilars as well as any Conjugates (PEG, Drug etc.)

- As of May 31, 2023
  29 of 32 products under review by the US FDA and the EMA are Antibody Products
  - 13 likely to be approved in 2023
  - 15 likely to be approved in 2024
  - 1 likely to be approved in 2025

Data and graphs derived from BDO's BPTG bioTRAK® database:
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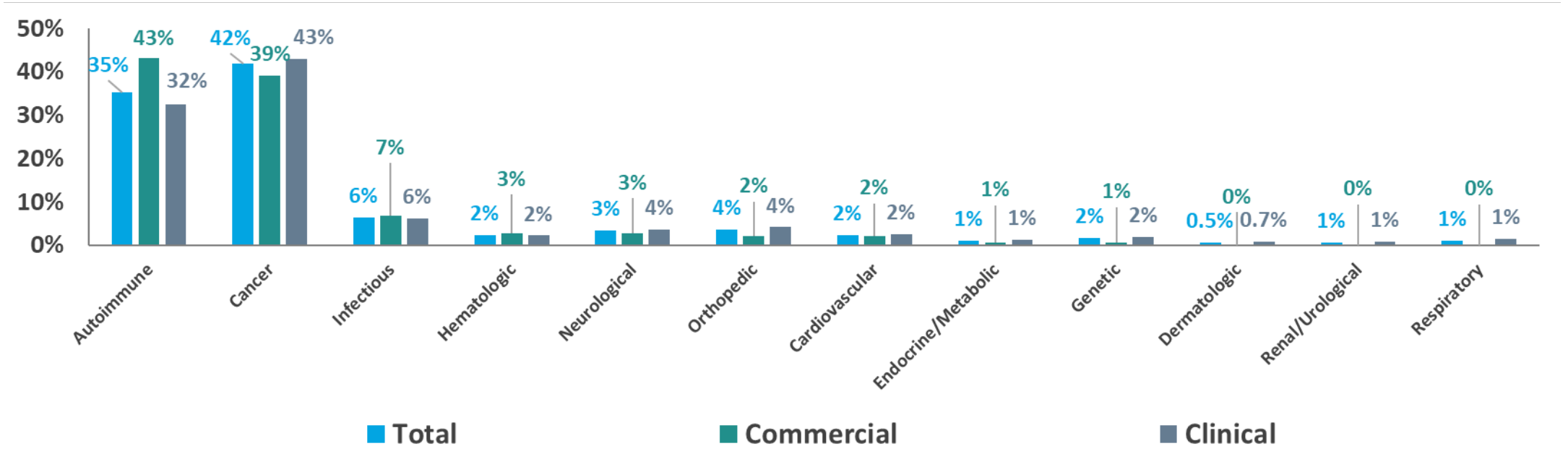
Monoclonal Antibodies by Indication

The graph shows all MAb products – Total, Commercial Only or Clinical Only by Indication Category



Data and graphs derived from BDO's BPTG bioTRAK® database:
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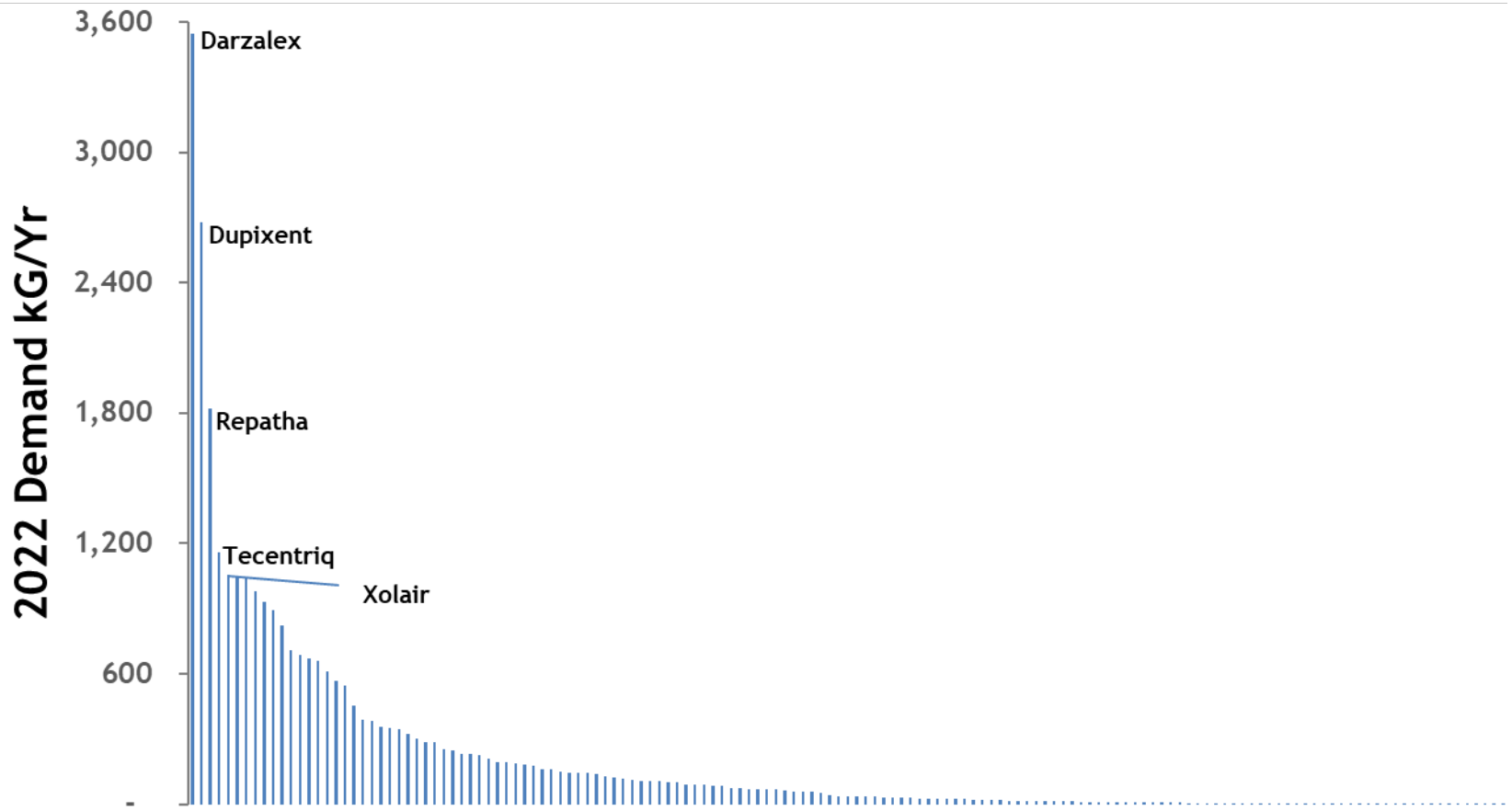
Demand (kg) for Antibody Products



- For the 156 approved (through 2022) therapeutic antibody products, the kg demand is shown
- Antibody products approved in 2023 are not included in this dataset
- Products recently approved may not have achieved full market penetration
- Ten products represent 50% of all demand
- Average kg demand: 206 kg
- Median kg demand: 34 kg

Data and graphs derived from BDO's BPTG bioTRAK® database:
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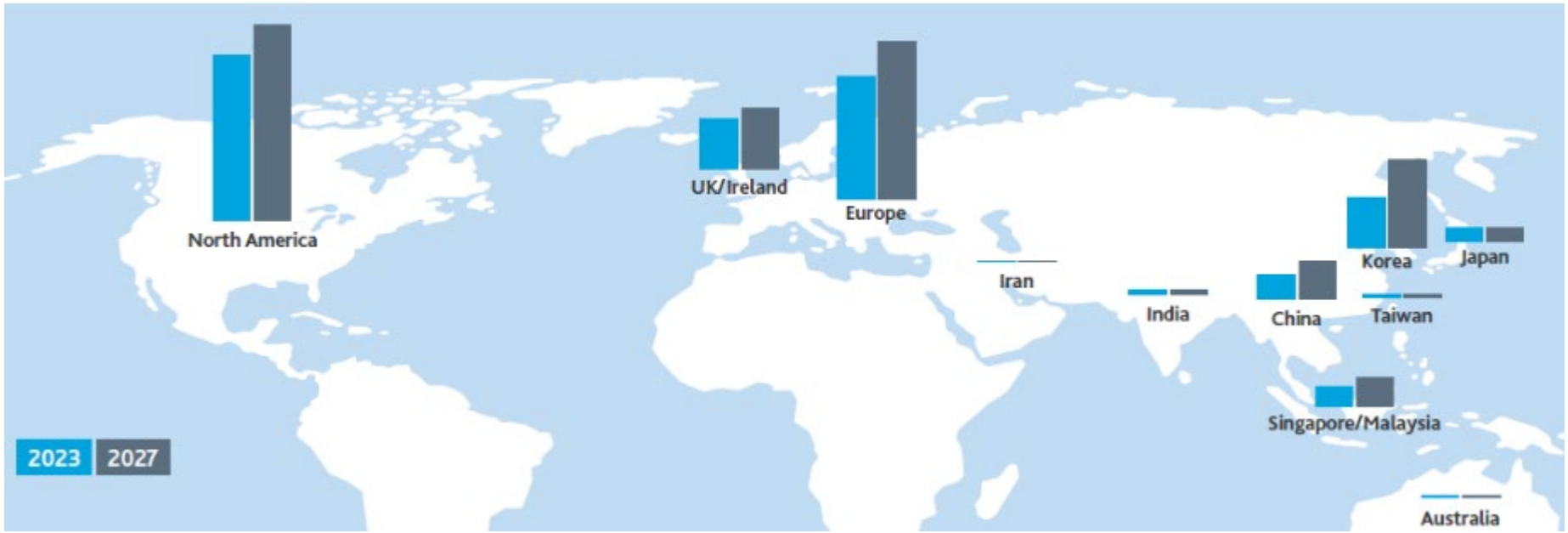
Biopharmaceutical Market Comparison of 2023/2027



Region	2023	2027
	Volume in '000s L	
North America	2,354	2,803
UK/Ireland	719	866
Europe	1,749	2,255
Korea	702	1,252
China	342	532
Singapore/ Malaysia	297	417
All Other Asia	309	315

Data and graphs derived from BDO's BPTG bioTRAK® database:
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Biopharmaceuticals Supply Trend

The Biopharma industry plans to build approximately 1.800.000 L of new capacity in the coming years for a 2027 total of just over **8.400.000** L

40 companies are planning to expand their Manufacturing capacity from 2023 to 2027, 5 of which are expanding >100kL

Examples:

- Fujifilm Diosynth Biotechnology is expanding ~480kL in Denmark, US and UK
- WuXi Biologics is projected to increase capacity by ~310kL in Singapore, China, the US and Ireland
- Samsung Biologics is expanding 180kL in South Korea
- Lonza is expanding ~140kL in Switzerland and the US
- Lotte Biologics, a new CMO is expanding ~140kL in South Korea and the US
- AGC Biologics doubling capacity in Denmark

Innovation in Biopharma is Driven by Biotech

Biotech Hubs in Europe

Similar Biotech clusters are found in many other parts of the world:

North America:

East and West-coast (eg Boston, San Francisco, San Diego, Washington, Raleigh-Durham, Montreal)

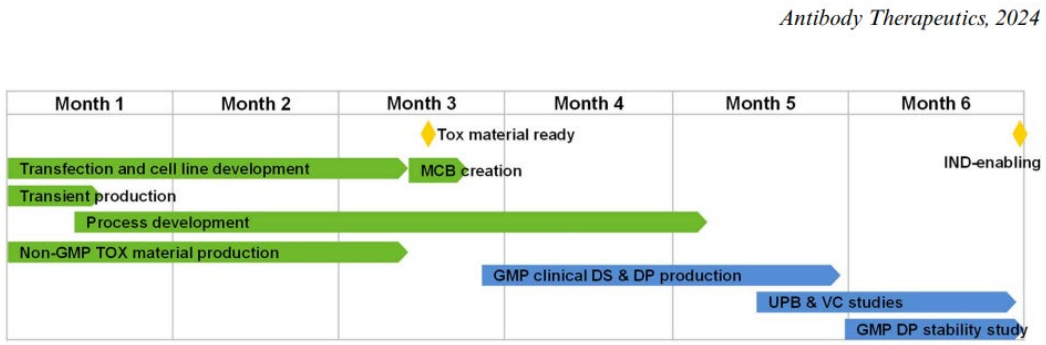
Asia (eg Shanghai, Beijing, Taiwan)

South America (eg São Paolo)

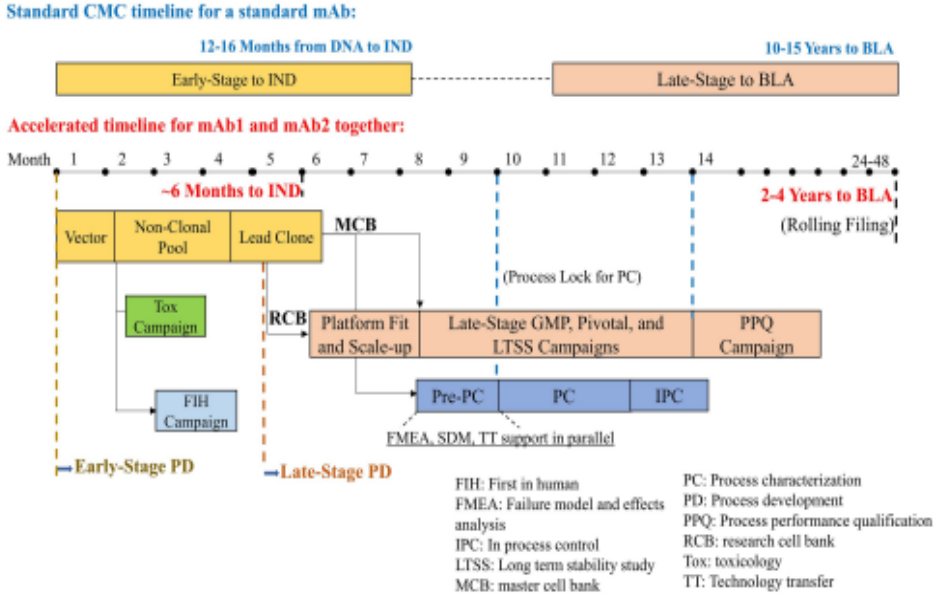
Accelerating Timelines and Streamlining Development Requirements:

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

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



Bristol Myers Squibb 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 during Covid: Regeneron, Catalent, AstraZeneca, Lilly, GSK…

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

Pre-clinical development timelines were reduced from **12-15 months to 6 months!**

Key instruments used to achieve this:

- IND enabling CMC/emergency use/conditional marketing authorization:
  - **Stable pool production – no production cell line cloning!**
  - **Highly integrated workflows (CMC/clinical)**
  - **Risk taking**
  - **Close interaction between industry and regulatory authorities**

Regulatory agencies were involved concurrently and they approved the novel concepts.

- Will they follow this trend also outside global crises?
- Is there a need to exchange the IND-enabling production cell with a ”clone” ?

Industrial Perspectives of Biologics Drug Supply

Future needs:

- Large amounts of drug substance – and small amounts for some products!
- Lower pricing of biologics
- Better quality control of drugs

?

Acknowledgements

BioProcess Technology Group at BDO

- Dawn Ecker

Biogen

- Technology Development
- Manufacturing Sciences

Boehringer Ingelheim

- BP Process Science Germany

Symphogen

- and multiple other colleagues from industry and academia working with

Cells vary in geno- and phenotype and all behave differently
- just like children

Thank you

Frederiksborg slot/Hillerød Castle, Denmark