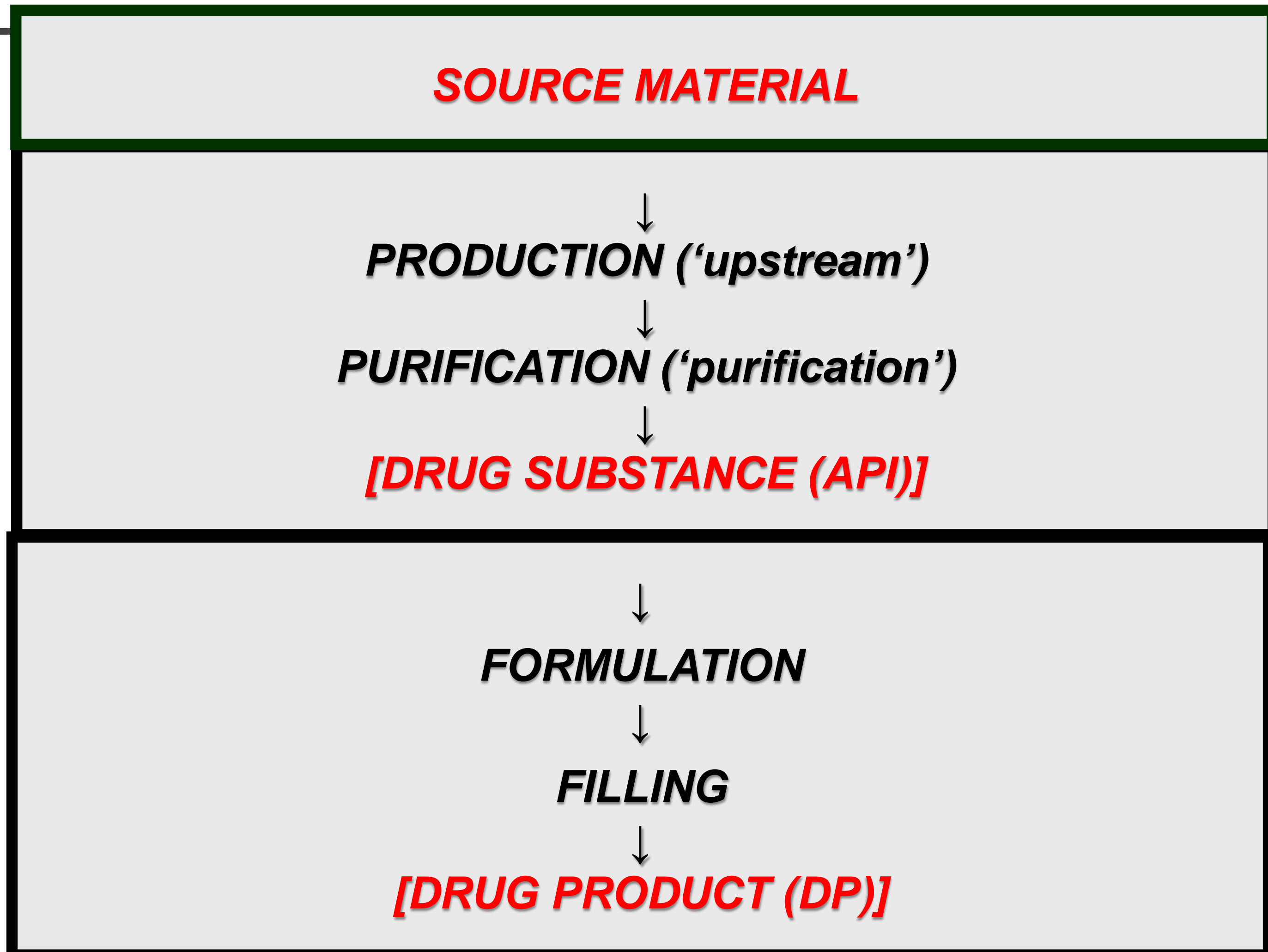
Course Outline

3. **Applying the CMC Risk-Managed Control Strategy Throughout the Entire Biopharmaceutical Manufacturing Process**

   - ✓ ***Walk through entire manufacturing process from source material → to drug product for a mAb***
     - *comparing FDA and EMA expectations*
     - *biologic vs chemical drug CMC regulatory requirements*
     - *risk-based decisions*
   - ✓ ***Comparing/contrasting a protein-based manufacturing process with a virus-/cell-based manufacturing process***

Basic Manufacturing Process Flow Diagram

Application of CMC Risk-Managed Control Strategy



SOURCE MATERIAL

Chemical drug*:** ***the starting material is a substance of defined chemical properties and structure, in which a significant structural fragment of the chemical is present

ICH Q11

Biologic: ***the source material is a biological substance that either contains already the desired biologic product or contains the genetic capability of producing the desired biologic product***

EC Directive 2001/83/EC of the European Parliament and Council, Concerning Community Code Relating to Medicinal Products For Human Use (October 2012)

Overview of the Manufacture of Biopharmaceutical API Types			
Manufacturing Process	Recombinant Protein/ Monoclonal Antibody	Genetically Engineered Virus	Genetically Engineered Cells
Source Material (contains the genetic elements)	Genetically engineered cell bank	Genetically engineered virus seed bank or plasmid(s) bank	Patient's cells + Genetically engineered virus
↓ Expression	Cell culture (protein induction)	Cell culture (virus transduction or plasmid(s) transfection)	Patient's cells (virus transduction)
↓ Purification	Chromatography to purify ***protein*** (removal of impurities)	Chromatography to purify ***virus*** (removal of impurities)	Filtration/washing to purify ***cells*** (removal of impurities)

Biologic Type	Source Material
Recombinant Proteins & Monoclonal Antibodies	*Master Cell Bank (MCB)*

Cell banks are the starting point for manufacture of biotechnological drug substances and some biological drug substances. In some regions, these are referred to as source materials; in others, starting materials. Guidance is contained in ICH Q5A, Q5B, and Q5D.

ICH Q11

(Step 1) Obtaining the basic genetic components

- ***Gene*** *– genetic material that contains the capability of producing the desired structure/product*

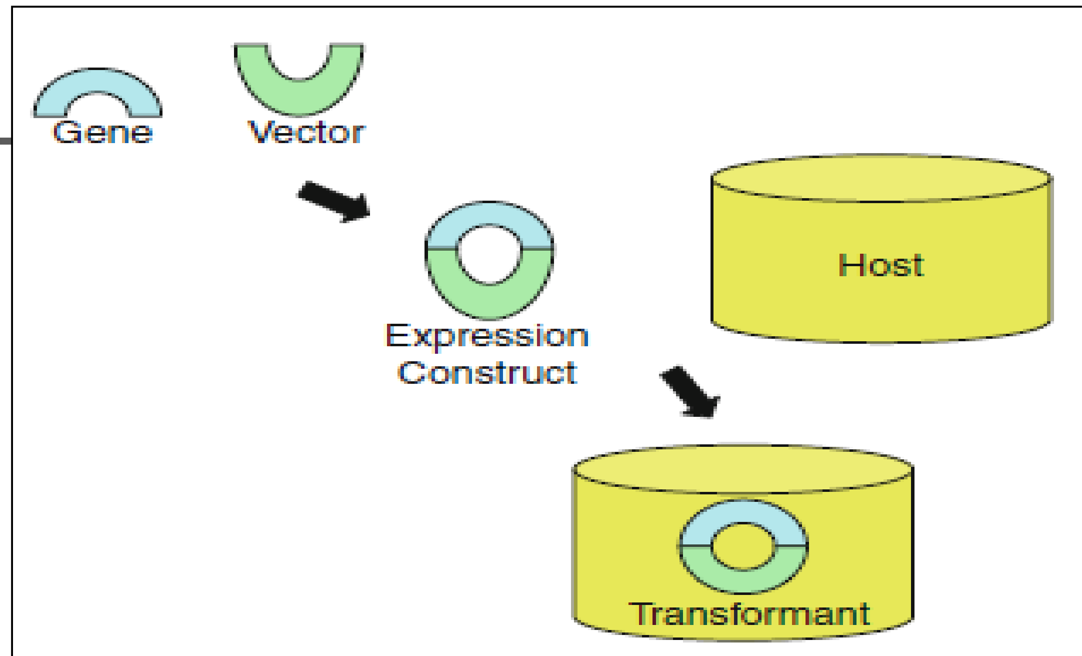
- ***Vector*** *– larger piece of DNA (e.g., plasmid, virus) that contains promoters, enhancers and other genetic pieces to allow the gene to function and survive within a foreign host*

  Expression construct *– gene inserted into vector (e.g., a DNA plasmid)*

- ***Host*** *– living cell into which the expression construct is to be inserted that provides the 'energy' to enable the gene to function*

Assembling the Recombinant Master Cell Bank

(Step 2) Developmental Genetics – putting the pieces together



- **Non-chemical transformation** (e.g., electroporation – high strength electric pulses to form transient holes in the cell membrane allowing the expression construct to enter the cell)
- **Chemical-based transfection** (e.g., liposomes that fuse with the cell membrane releasing the expression construct into the cell)
- **Virus transduction** (e.g., viruses used as carriers of the expression construct into the cell)

Transformants ***– thousands upon thousands of recombinant cells***

Cloning ***– selection of a single recombinant cell that contains the desired functioning expression construct***

Cell expansion ***– under defined cell culture conditions, of the selected cloned cell that possesses the potential for producing the desired biopharmaceutical***

Cell Substrate

Assembling the Recombinant Master Cell Bank

(Step 3) Laying Down the Cell Bank

Cell Substrate

Master Cell Bank (MCB)

the expanded cell substrate Is aliquotted into multiple containers and stored under defined long-term conditions

Working Cell Bank (WCB)

An aliquot of the MCB is grown under defined cell culture conditions and then aliquotted into multiple containers and stored under defined conditions

- ***One MCB or WCB aliquot is typically needed per production batch***
- ***Typical cell bank size – 200-250 aliquots***
- ***200 MCB aliquots can yield 200 x 200 WCB aliquots (~40,000 batches)***

(MCB, MVB, MPB)

- ***Homogeneous (equivalent aliquots)***
- ***Fully characterized***
- ***Free of adventitious agents and undesired impurities***
- ***Readily available when needed for manufacturing***

***Three* myths about Recombinant MCBs!**

"Myth" - a traditional or legendary story, with or without a determinable basis of fact, that explains some practice

Myth #1

A Master Cell Bank used in clinical studies is always acceptable for commercial manufacturing!

To initiate human clinical studies – MCB limitations

1 of 2: minimum regulatory authority expectations

Source, history and generation of the cell substrate

A brief description of the source and generation (flow chart of the successive steps) of the cell substrate, analysis of the expression vector used to genetically modify the cells and incorporated in the parental / host cell used to develop the Master Cell Bank (MCB), and the strategy by which the expression of the relevant gene is promoted and controlled in production should be provided, following the principles of ICH Q5D.

Cell bank system, characterisation and testing

A MCB should be established prior to the initiation of phase I trials. It is acknowledged that a Working Cell Bank (WCB) may not always be established.

EMA Guideline on the Requirements for Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trials (September 2018)

To initiate human clinical studies – MCB limitations

2 of 2: regulatory authority reviewers do not catch everything

Although CDER acknowledges its review responsibilities,
it does not have unlimited resources to review all submissions with the highest level of scrutiny in short time frames.
CDER review staff must prioritize their workload and evaluate individual submissions in the context of their place in drug development…
review of a new IND focuses primarily on safety….

FDA CDER Manual of Policy and Procedures (MAPP): MAPP 6030.9 – Good Review Practice: Good Review Management Principles and Practices for Effective IND Development and Review (April 2013)

Patient Safety Focus of Review

1 of 3: absence of adventitious agents of concern

- **Prions – TSEs**
  - Prevented through risk minimization strategy in choices for raw materials used to prepare bank
- **Viruses – insect/animal/human cell lines**
  - Extensive viral safety testing of bank; $$$
- **Mycoplasmas – insect/animal/human cell lines**
  - 28 day testing of bank
- **Bacteria/Fungi – all cell lines**
  - Culture purity testing of bank (if bacterial/yeast)
  - Sterility testing of bank (if animal/human)

ICH Q5D

Patient Safety Focus of Review

2 of 3: absence of non-host cells

The purity of cell substrates can be compromised through contamination by cell lines of the same or different species of origin. The choice of tests to be performed depends upon whether opportunities have existed for cross-contamination by other cell lines. In some cases, it may be necessary to maintain growing cultures of different cell lines in the same laboratory. During procedures in cell banking where open manipulations are performed, care should be taken to ensure that simultaneous open manipulations of other cell lines are avoided to prevent cross-contamination. Whenever another cell

ICH Q5D

Where was your genetic engineering done? In R&D

Absence confirmed by documentation of procedural controls

Patient Safety Focus of Review

3 of 3: identity (characterization) of genetic components

- **Gene Authentication**
  - DNA sequencing to confirm correct nucleotide sequence
  - Protein sequencing to confirm correct amino acid sequence from DNA
- **Vector Authentication**
  - DNA sequencing to confirm correct regulatory/control elements
  - Restriction enzyme mapping of vector
- **Host Authentication**
  - DNA fingerprinting

ICH Q5B

ICH Q5D

- ***Patient safety continues to remain the primary regulatory evaluation of the MCB***
- ***<u>But now</u>, the MCB is also thoroughly reviewed to determine if it can meet the expectations for a stable, continuous, homogenous source for ongoing future manufacturing***

***Gene Construct** – A detailed description of the gene which was introduced into the host cells, including both the cell type and origin of the source material, should be provided…The complete nucleotide sequence of the coding region and regulatory elements of the expression construct, with translated amino acid sequence, should be provided, including annotation designating all important sequence features.*

***Vector** – Detailed information regarding the vector and genetic elements should be provided, including a description of the source and function of the component parts of the vector, e.g. origins of replication, antibiotic resistance genes, promoters, enhancers.*

***Final Gene Construct** – A detailed description should be provided of the cloning process which resulted in the final recombinant gene construct. The information should include a step-by-step description of the assembly of the gene fragments and vector or other genetic elements to form the final gene construct.*

FDA Guidance For Industry For the Submission of Chemistry, Manufacturing , and Controls Information For a Therapeutic Recombinant DNA-Derived Product or a Monoclonal Antibody Product For In Vivo Use (August 1996)

Two Case Examples of MCB Concerns

- ***Lack of identify of genetic components***
- ***Lack of confirmed absence of adventitious virus***

Discovered MCB concern after clinical development is completed

lack of identity of genetic components

Recombinant Protein produced by Recombinant Carrot Cells

You have provided nucleic acid sequencing data. indicating that only ___ of the sequenced clones had the expected deoxyribonucleic acid (DNA) sequence, with some of the changes in DNA sequence altering the protein sequence.

You attributed this result to matrix effects and polymerase chain reaction (PCR) artifacts but provided no data to support this conclusion. Additionally, no information was provided demonstrating that the protein coding sequence is maintained during culture to the end of production.

These results suggest that the gene sequences in the master cell bank are not identical to the expression construct gene sequence, inconsistent with ICH Q5B.

FDA Drugs – Search Drugs@FDA – FDA Approved Drug Products: Elelyso (Taliglucerase Alfa) – Approval History, Letter, Reviews and Related Documents – Administrative and Correspondence Documents – BLA Information Request Letter (October 28, 2010)

Discovered MCB concern after clinical development is completed

lack of confirmed absence of adventitious virus

Recombinant Protein produced by CHO Cells

The master file you reference ___ does not provide sufficient information to assess the adequacy of virus testing of this human sourced component and your master cell bank has not been tested for the presence of any human viruses.

This raises a concern that human virus may be present in your cell bank and this could impact the safety of your final drug product.

Therefore, provide a risk assessment and relevant data (literature reference, etc.) on human virus infection and propagation in your CHO-K-1 cell line... Based on this information, you should provide a risk assessment and propose and justify a strategy to test your master cell bank for the most relevant human viruses, or justify why testing for the presence of human viruses is not necessary.

FDA Drugs – Search Drugs@FDA – FDA Approved Drug Products: Vimizim (Elosulfase Alfa) – Approval History, Letter, Reviews and Related Documents – Administrative and Correspondence Documents – BLA Information Request Letter (August 02, 2013)

Current Red Hot MCB Issue with Regulatory Authorities

PROOF OF MCB CLONALITY

***MCB (Master Cell Bank)*. An aliquot of a single pool of cells which generally has been *prepared from the selected cell clone* under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks**

ICH Q5D (1997) *EC GMP Annex 2 (2018)*

THE CONCERN: Non-clonal cell bank can give rise to outgrowth of a subpopulation of cells that generate products with different CQAs

Transformant cells → Cloning → Cell Substrate → MCB

1000's of 1000's *1 transformed cell*

World Health Organization (WHO)

recommended approach to cloning!

In the process of cloning a cell culture, single cells should be selected for expansion. The cloning procedure should be carefully documented, including the provenance of the original culture, the cloning protocol, and reagents used. Cloning by one round of limiting dilution will not necessarily guarantee derivation from single cells; additional subcloning steps should be performed. Alternatively or in addition to limiting dilution steps the cloning procedure can include more recent technology such as single cell sorting and arraying, or colony picking from dilute seeds into semi-solid media. In any case, the cloning procedure should be fully documented, accompanied by imaging techniques and/or appropriate statistics. For proteins derived from transfection with recombinant plasmid DNA technology a single, fully documented round of cloning is sufficient provided product homogeneity and consistent characteristics are demonstrated throughout the production process and within a defined cell age beyond the production process.

WHO Evaluation of Animal Cell Cultures as Substrates TR978 (2013)

NOTE: strong emphasis on documentation done in R&D!

USP <1042> Cell Banking

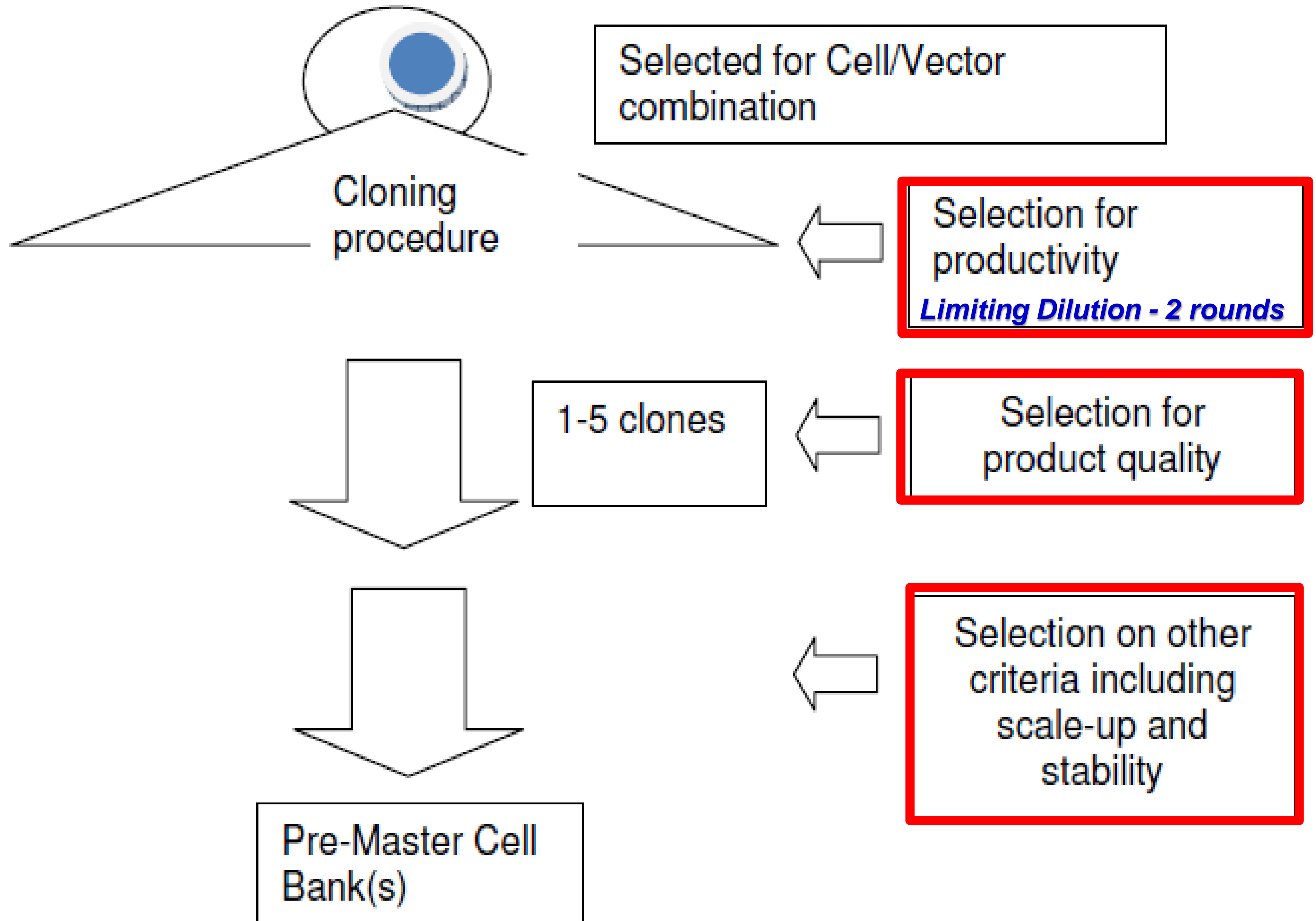
cloning must be documented when it is done!

LIMITING DILUTION CLONING

Limiting dilution cloning (LDC) is a procedure whereby cells are plated at a low density, ideally <0.5 cells/well in a 96-wellplate, with the aim of obtaining only 1 cell in a well from which progeny can grow. Some wells will be devoid of cells. This is achieved by preparing a set of increasingly greater dilutions of the non-clonal starting population and visually verifying the number of cells initially deposited per well.

Two rounds of LDC are recommended if manufacturers want to establish a clonal cell line, particularly in the absence of additional supporting technology, to ensure monoclonality (e.g., imaging).
Two rounds of LDC provide an approximately 99% probability that the cell line will be monoclonal. However, it is a time-consuming process and can take up to 12 months to complete.

WHO – illustration of three essential screens in clone selection



Improved rapid and more sensitive techniques for FIRST STEP: detection (heightened imaging) and evaluating productivity of clones

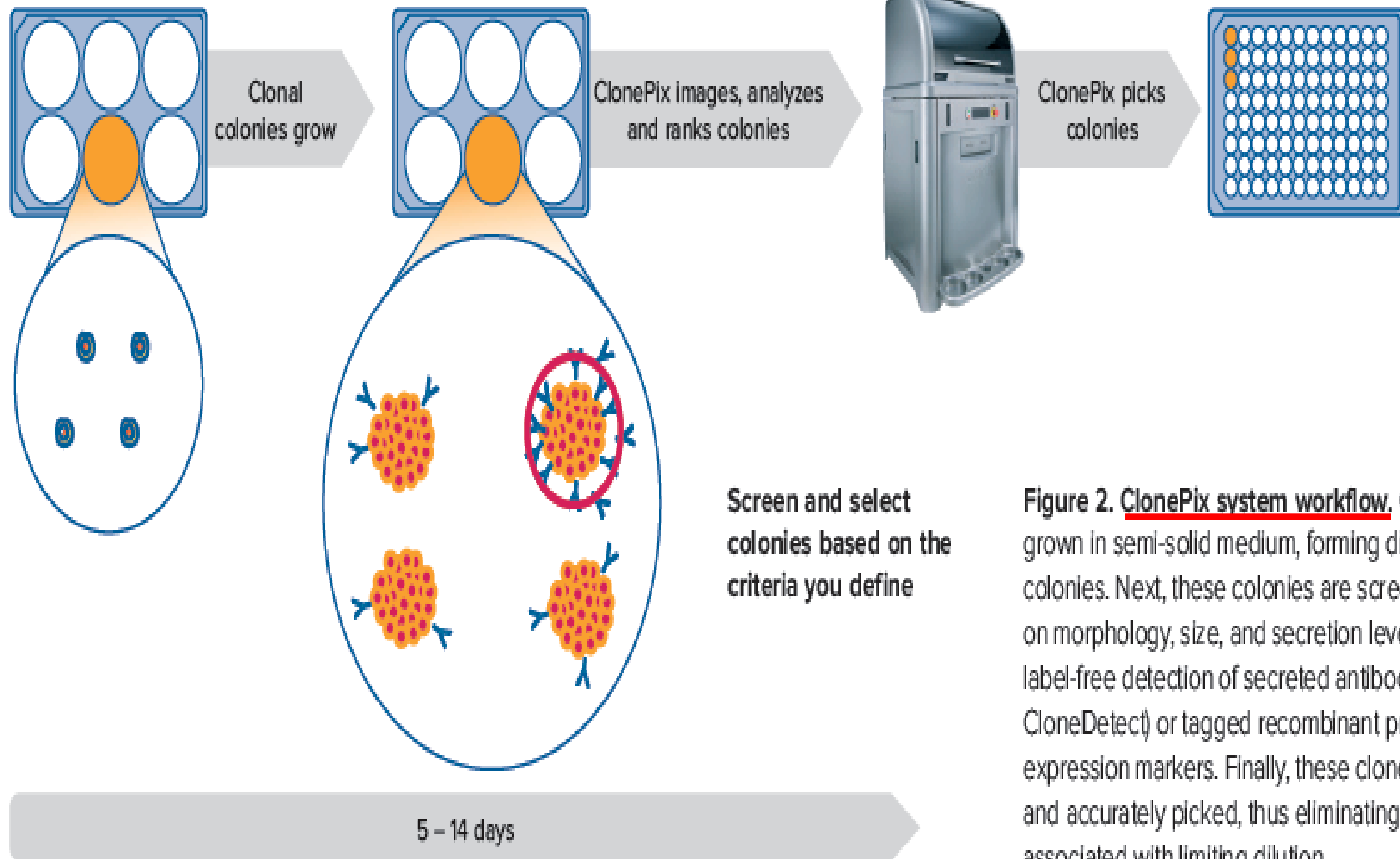
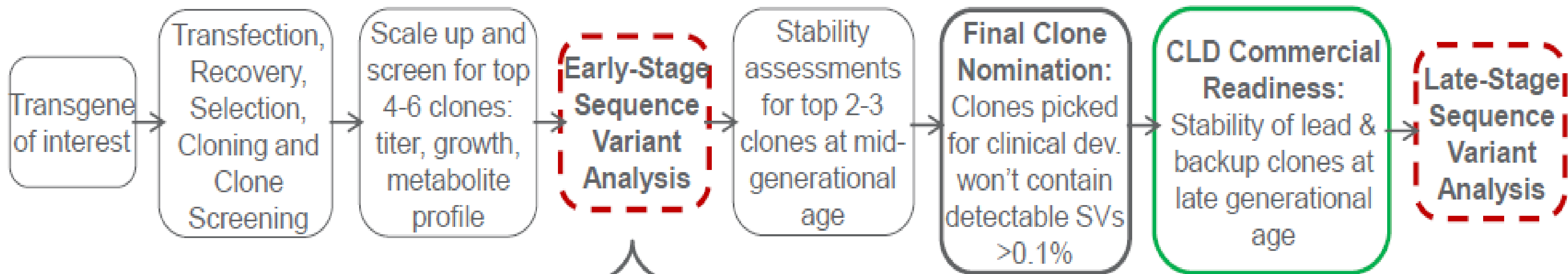


Figure 2. ClonePix system workflow. Cells are grown in semi-solid medium, forming discrete clonal colonies. Next, these colonies are screened based on morphology, size, and secretion level using label-free detection of secreted antibodies (such as CloneDetect) or tagged recombinant proteins and expression markers. Finally, these clones are ranked and accurately picked, thus eliminating errors associated with limiting dilution.

Improved selection tools for SECOND STEP: evaluating product quality of clones



Redacted: Early SV Analyses w/MS-PQ

AMBR

- **PQ: Glycoforms / reducible species:** LC/MS intact mAb profiling (≥1%) — **Not needed**
- **High-level SV/PQ screen:** LC/UV/MS subunit mapping (≥1%) — **Not needed**
- **DNA / mRNA mutation screen:** expanded, extensive clonal sequencing (≥2%) — **Switch to NGS**
- **Low-level SV/PQ screen:** trypsin LC-MS/MS w/ SV bioinformatics (>0.1%)

→

Upgraded: Early SV Analyses

AMBR

- **Automated DNA / mRNA mutation analysis:** next-gen sequencing (≥0.5%)
- **Automated low-level SV screen:** trypsin LC-MS/MS w/ bioinformatics (>0.1%)
- **Bio-PQ:** Titer, SEC (HMMS), rCGE (fragments), iCE (charge distribution), gene copy number, N-glycan profile

Action levels are in parenthesis

Product Quality Attributes		MCB	Clone 1 (%)	Clone 2 (%)	Clone 3 (%)	Clone 4 (%)	Clone 5 (%)	Clone 6 (%)
Heavy Chain N-Terminal Heterogeneity [1]	Unmodified	97.0	97.6	98.0	98.1	97.9	97.7	97.7
	Pyroglutamic acid	2.5	2.4	2.0	1.9	2.1	2.3	2.1
	-3VHS	0.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2
Heavy Chain C-Terminal Heterogeneity [1]	Unmodified	92.3	87.9	81.7	90.3	83.9	92.0	89.1
	Amidated proline	**3.7**	**0.7**	**0.7**	**0.8**	**0.7**	**0.4**	**0.6**
	C-terminal lysine	**3.5**	**8.9**	**12.9**	**7.0**	**11.5**	**5.9**	**8.2**
Light Chain N-Terminal Heterogeneity [1]	Unmodified	93.6	88.4	89.5	89.3	87.3	88.1	89.2
	-3VHS	N/A	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
	des – 1SYE	**4.0**	**11.3**	**10.4**	**10.3**	**12.4**	**11.3**	**10.6**
N-Glycans [2]	G0F	82.3	66.4	65.7	79.8	66.6	69.0	70.4
	G1F	7.3	22.6	21.4	15.3	23.7	24.9	21.2
	G2F	0.3	2.1	2.2	0.9	2.2	2.3	1.8
	G0	5.0	2.9	2.3	2.3	2.0	1.7	1.7
	G0F minus GlcNAc	1.0	1.1	2.1	0.3	1.0	< 0.1	0.6
	Man5	1.3	2.9	3.8	0.2	2.6	0.5	1.2
	Aglycosylated	2.8	2.0	2.5	1.2	1.9	1.6	3.1
Trisulfides [3]	One trisulfide	**ND**	**35**	**36**	**29**	**31**	**Trace**	**ND**
	Two trisulfides	**ND**	**17**	**20**	**11**	**13**	**ND**	**ND**

WORLDWIDE RESEARCH & DEVELOPMENT
BioTherapeutics Pharmaceutical Sciences

ND = not detected
N/A = not applicable

1. *Determined by LC/MS/MS-peptide mapping*
2. *Determined by LC/MS-3-part subunit analysis*
3. *Determined by LC/MS – intact mAb analysis*

Reviewer Considerations for Clonality at the IND stage

FDA

- At the IND stage, reviewers will do a initial assessment of the information provided about the clonality of the MCB. If significant deficiencies are noted, then the appropriate comments will be communicated.
- Lack of assurance of clonality is **not** necessarily a hold issue.

Considerations at the BLA stage

FDA

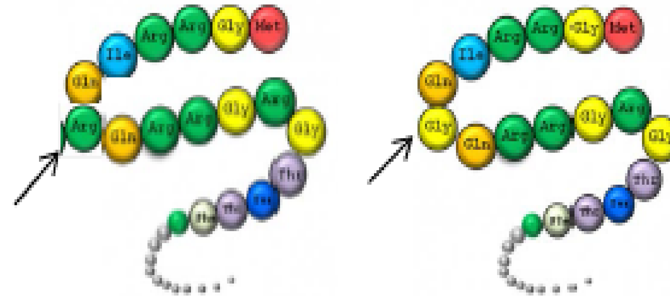
- Adequate assurance of clonality should be provided at the time of the BLA submission.
- Having low assurance of clonality of the MCB at the time of licensure does ***not*** necessarily preclude approvability of the application.
- Augmentation of the control strategy could be an acceptable approach to managing a non-clonal MCB for licensure.

R. Novak, CDER, WCBP 2017

Monoclonality

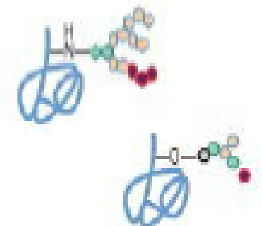
Dr. Una Moore
Health Products Regulatory Authority, Ireland

16th April 2014.



The DS could be a mixture e.g.

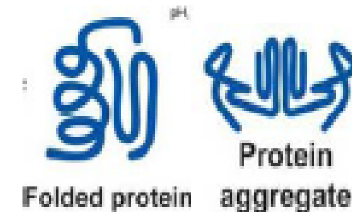
Different amino acid sequence



Different post translational modifications e.g. N or O linked glycosylation

Different impurity profile e.g. deamidation, oxidation, aggregation profile

Different functional activity



Consequences:

Complete physical, chemical and functional characterisation to confirm same DS

Investigations into the source of DS/DP (i.e. which clone) used in each CT

Possible repetition of CTs, rejection of MAH

Monoclonality should be confirmed before phase 1 CT

- ***Deny approval***
- ***Require additional studies to confirm clonality***
- ***Augment the control strategy***

- Some strategies that have been implemented:
  - Adding additional specifications (LC-MS/MS for Sequence Variants, Glycosylation despite not impacting MOA, etc.)
  - Tighter limits on the limit of in vitro cell age
  - Establishing additional critical process parameters (growth parameters escalated to CPP)
  - Trending and Statistical Process Control
  - Additional risk assessment for changes in critical raw materials (media, components, etc.)
  - Tighter controls for re-qualification of a new WCB

R. Novak, CDER, WCBP 2017

Three Case Examples of MCB Clonality Issues

- ***mAb – SP2/0 murine cells – orphan drug designation for a rare pediatric disease***
- ***mAb – CHO cells – orphan drug designation, Fast Track, Breakthrough Therapy designation***
- ***mAb – CHO cells – Fast Track***

In each case, the manufacturer was assigned a postmarketing commitment to complete the clonality testing

Monoclonal Antibody produced by Murine SP2/0 Cells

Insufficient information has been provided to date to support the clonality of the production cell line… You need to provide available information on the cloning procedures performed by NCI as well as detailed information on the cloning procedures performed… Provide a calculation on the probability of clonality of the production cell line along with information on how the probability was calculated.

Conduct studies to further characterize the Unituxin master cell bank (MCB) and to confirm the monoclonality of the MCB.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Unituxin (Dinutuximab) – Approval History, Letters, Reviews and Related Documents – Administrative and Correspondence Documents – CMC Information Request (August 06, 2014)

Discovered MCB concern about proof of clonality

after clinical development is completed

Monoclonal Antibody produced by CHO

A formal cloning procedure was conducted only once. Therefore, there is residual uncertainty for the monoclonality of burosumab MCB.
The specifications for burosumab drug substance and drug product are acceptable to ensure adequate quality and safety for the initial marketed product. Assurance of the monoclonality of the burosumab MCB will reduce the risk of the generation of product variants and ensure the consistency of product quality throughout the product life cycle.

Conduct studies to further characterize the burosumab master cell bank (MCB) and to support the monoclonality of the MCB.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Crysvita (Burosumab-twza) – Approval History, Letters, Reviews and Related Documents – Other Reviews – PMR/PMC Development Template: Product Quality (CMC) – PMC #1 (April 17, 2018)

Monoclonal Antibody produced by CHO

Testing for the identity, safety and genetic stability of the cell bank was performed. However, as the cell cloning procedure did not provide a high assurance of clonality of the master cell bank. The cell line genetic stability and product quality data submitted to the BLA provide assurance that the current manufacturing process is not impacted by the clonality of the cell bank; however it did not address the impact of different manufacturing conditions throughout the product life cycle.

To address this issue the Applicant agreed to perform additional testing of the master cell bank to support clonality as a postmarketing commitment.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Zinplava (Bezlotoxumab) – Approval History, Letters, Reviews and Related Documents – Administrative and Correspondence Documents – Summary Review (October 21, 2016)

Question: How effective is your archival system to retrieve developmental genetic documents/notebooks related to the MCB preparation from 7-10 years ago?

At Phase 1 Start	**For Market Approval**
Documentation (*brief* description)	**Documentation** (*detailed* description)

A Suggestion

Prepare the detailed description report when the MCB is prepared!
(this will ensure that any concerns are noted early)

Summarize this document for the Phase 1 filing; archive the original detailed report until needed for the market dossier submission!

If brave, submit the detailed report In the Phase 1 regulatory submission (so that it can be readily located in the future)

MCB Inventory Management Concerns

raised <u>only</u> at market approval stage

Storage containers should be sealed, clearly labelled and kept at an appropriate temperature. A stock inventory must be kept. The storage temperature should be recorded continuously and, where used, the liquid nitrogen level monitored. Deviation from set limits and corrective and preventive action taken should be recorded.

It is desirable to split stocks and to store the split stocks at different locations so as to minimize the risks of total loss.

Once containers are removed from the seed lot / cell bank management system, the containers should not be returned to stock.

EC GMP Annex 2 (2018)

1) ***Must have an acceptable cell bank inventory level***
2) ***Need to have cell bank long-term storage stability***
3) ***Must have a catastrophic event plan for the cell bank***

Manufacturers should describe their strategy for providing a continued supply of cells from their cell bank(s), including the anticipated utilization rate of the cell bank(s) for production, the expected intervals between generation of new cell banks,....

ICH Q5D

Be cautious, assume worst case (double your calculated utilization rate!)

What is an acceptable MCB/WCB inventory level? 20 years, 10 years, ?

2) Cell bank long-term storage stability

Evidence for banked cell stability under defined storage conditions will usually be generated during production of clinical trial material from the banked cells. Available data should be clearly documented in the application dossiers, plus a proposal for monitoring of banked cell stability should be provided.

The proposed monitoring can be performed at the time that one or more containers of the cryopreserved bank is thawed for production use, when the product or production consistency is monitored in a relevant way, or when one or more containers of the cryopreserved MCB is thawed for preparation of a new WCB (and the new WCB is properly qualified), as appropriate.

ICH Q5D

Since few MCB aliquots are thawed to prepare a new WCB, when was the last time you checked the stability of the MCB?

(A WCB stability timepoint is obtained every time a WCB is thawed to initiate a cell culture batch)

So how frequent should the MCB be tested for stability?

One answer

- ***There is no regulatory authority guidance on the frequency of stability testing for a MCB, so consultants have typically recommended every 4-5 years***
- ***However, the FDA indicated their preference on the MCB frequency of stability testing in a communication to Genentech during the market approval of the CHO-produced monoclonal antibody, Perjeta (pertuzumab):***

Conduct stability studies of the Master Cell Bank at more frequent intervals than the currently proposed 10 years. Submit Interim Reports every four years and the Final Report after 20 years.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Perjeta (Pertuzumab) – Approval History, Letters, Reviews and Related Documents – Market Approval Letter (June 08, 2012)

3) Cell bank catastrophic event plan

What if the unthinkable happens?

To ensure continuous, uninterrupted production of pharmaceuticals, manufacturers should carefully consider the steps that can be taken to provide for protection from catastrophic events that could render the cell bank unusable. Examples of these events include fires, power outages and human error. Manufacturers should describe their plans for such precautions; for example, these may include redundancy in the storage of bank containers in multiple freezers, use of back-up power, use of automatic liquid nitrogen fill systems for storage units, storage of a portion of the MCB and WCB at remote sites, or regeneration of the MCB.

ICH Q5D

Manmade/natural catastrophes

fires, floods, ice storms, monsoons, earthquakes
hurricanes (e.g., Hurricane Maria – Puerto Rico 2017)

UCERF3

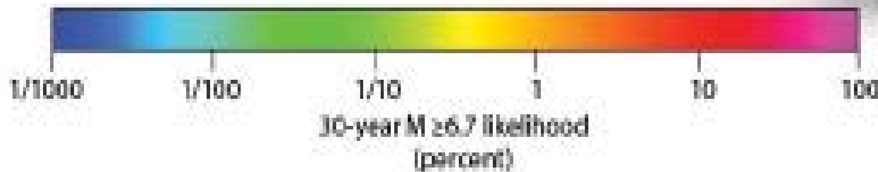
Uniform California Earthquake Rupture Forecast (Version 3)

Three-dimensional perspective view of the likelihood that each region of California will experience a magnitude 6.7 or larger earthquake in the next 30 years (6.7 matches the magnitude of the 1994 Northridge earthquake, and 30 years is the typical duration of a homeowner mortgage).

San Francisco region

Los Angeles region

State boundary



Faults are shown by the rectangles outlined in black. The entire colored area represents greater California, and the white line across the middle defines northern versus southern California. Results do not include earthquakes on the Cascadia Subduction Zone, a 750-mile offshore fault that extends about 150 miles into California from Oregon and Washington to the north.

Myth #1 Debunked

Myth #2

Exchanging out a Master Cell Bank during clinical development is not a major risk

GMP Compliance Reasons

- Lack of documentation on preparation of existing MCB
- Insufficient MCB inventory

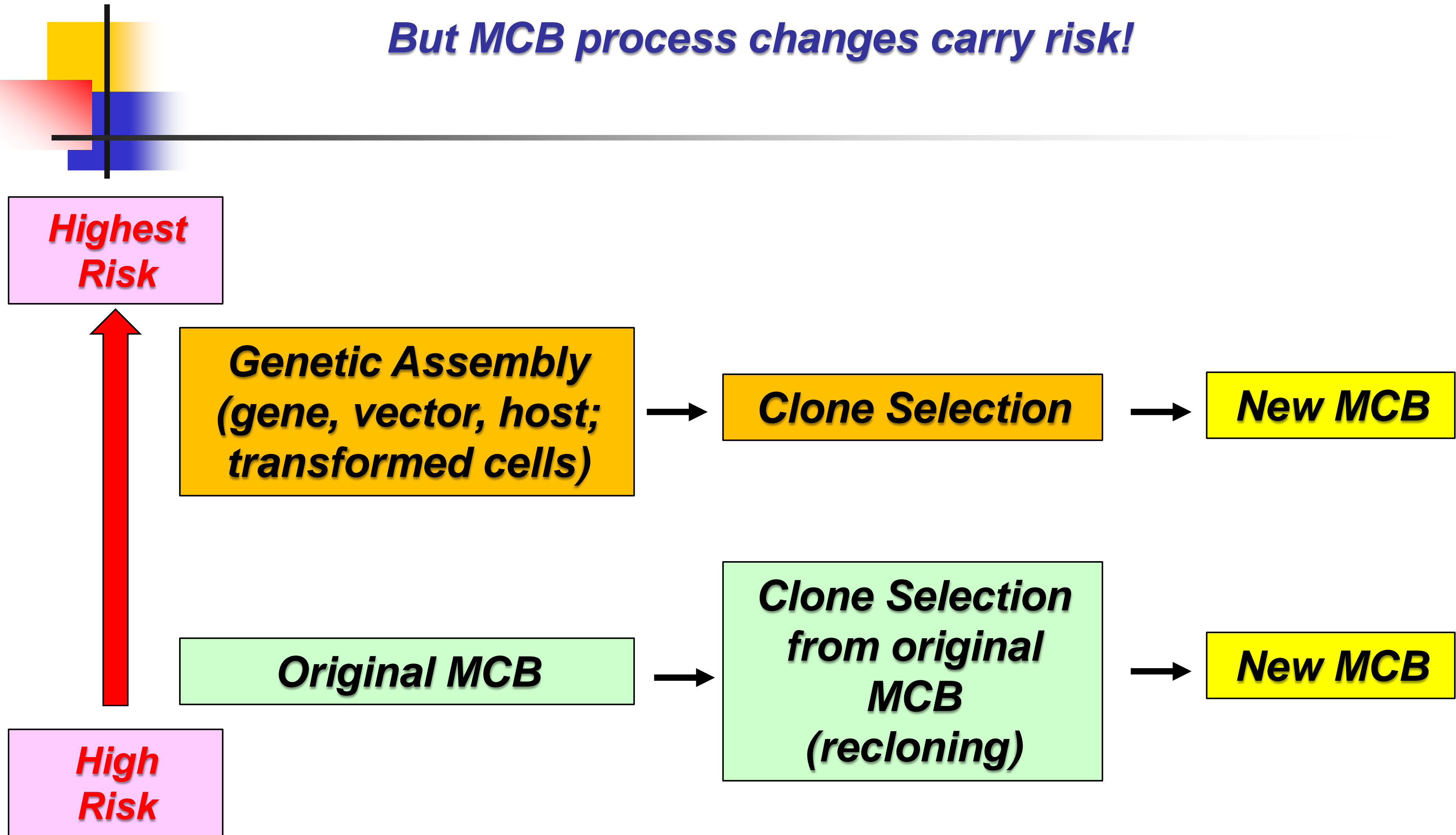
Quality Reasons

- Safety concern (e.g., mixed culture, contamination)
- Instability of existing frozen MCB

Manufacturing/Business Reasons

- Increases in product productivity
- Concern of clonal scale-up stability

But MCB process changes carry risk!

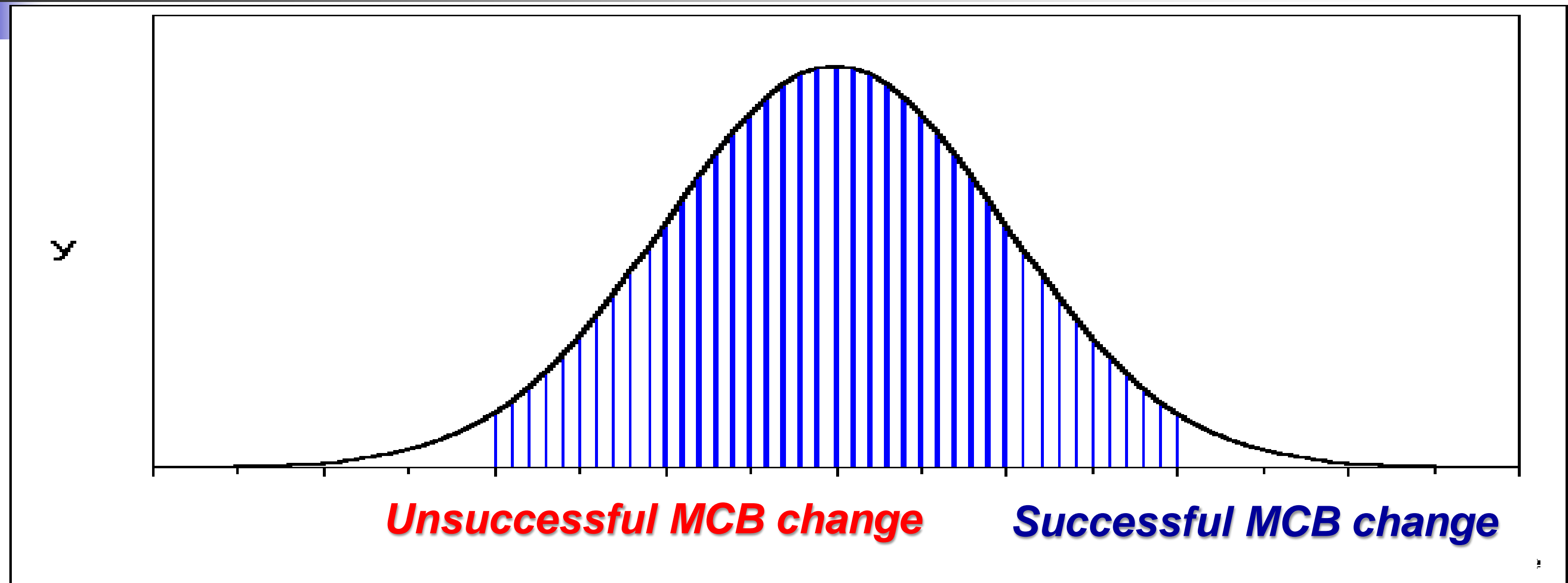


Despite the high risk, manufacturers have successfully replaced MCBs during clinical development

Marketed Biopharmaceutical	Successful MCB replacement during clinical development
Yervoy (ipilimumab) monoclonal antibody (May 2011)	A hybridoma clone, produced anti-CTLA-4 antibody, was selected and its product was used in Phase I clinical studies (Process A). For Phase II clinical studies and beyond, a recombinant CHO cell line was developed which expressed the same antibody sequence produced by the hybridoma
Lemtrada (alemtuzumab) monoclonal antibody (June 2013)	Alemtuzumab is produced in a Chinese Hamster Ovary (CHO) cell line… MCB1 was used to produce WCBs that produced clinical trial material. After the production of MCB1, a second MCB (MCB2) was prepared from a subclone of MCB1 to improve stability. MCB2 was fully characterized and is the source of all WCBs utilised for commercial production.

as reported in EMA EPARs

But what about MCB changes that were *not* successful?



Failures are 'proprietary'!

(issues rarely come 'to the light')

Myth #1 Debunked

A master cell bank that is considered acceptable for starting Phase 1 clinical trials will not necessarily be acceptable for manufacturing commercial biological products!

Myth #2 Debunked

Exchanging out a Master Cell Bank during clinical development is doable, but a major risk!

Myth #3

Working cell banks are never a problem!

At the clinical development stage

As for any process change, the introduction of a WCB may potentially impact the quality profile of the active substance and comparability should be considered.

EMA Guideline on the Requirements for Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trials (September 2018)

At the market approval stage

Qualification of the WCB will include
- *safety testing,*
- *an evaluation of the growth of WCB cultures relative to the growth of Master Cell Bank (MCB) cultures,*
- *testing of end of production cells generated from the commercial scale process, and*
- *a comparability assessment that includes the first three lots manufactured from the WCB using the commercial process.*

One lot manufactured using the commercial process will be placed on a stability protocol and the data will be submitted in the subsequent BLA annual reports.

The WCB qualification report will be submitted in a prior approval supplement.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Unituxin (Dinutuximab) – Approval History, Letters, Reviews and Related Documents – Market Approval Letter (March 10, 2015)

Although a rare event, Working Cell Banks (WCB) can create a major problem with manufacture of a recombinant protein or mAb

case example: Genentech – Perjeta (pertuzumab) – pre-approval inspection

In addition, while inspecting the facility, we discovered that the Sponsor was experiencing serious issues with the thaw and subsequent propagation of cells from WCB__ used to manufacture pertuzumab. At the time of inspection, the root cause investigation was ongoing and no root cause had been identified, although data suggested instability of WCB …

The 483 items cited on this inspection could generally be classified as VAI (voluntarily action indicated), but the deviation and follow up data supplied from the firm related to their inability to successfully thaw and grow cultures from their working cell bank lead us to concur with the recommendation to withhold on this application by Division of Monoclonal Antibodies.

FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Perjeta (Pertuzumab) – Approval History, Letters, Reviews and Related Documents – Chemistry Review – Product Quality Review Data Sheet (May 31, 2012)

In order to obtain market approval for their monoclonal antibody, Genentech was required by the FDA to carry out three concurrent WCB process validation plans:

1. ***manufacture the monoclonal antibody directly from the MCB***
2. ***develop a new WCB and start manufacturing from that one***
3. ***modify the cell growth process downstream from the WCB***

The WCB problem was eventually resolved
(but Genentech has not disclosed
what was the actual problem, or the solution)

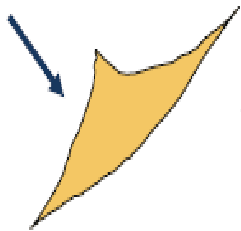
FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Perjeta (Pertuzumab) – Approval History, Letters, Reviews and Related Documents – Market Approval Letter (June 08, 2012)

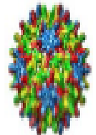
Myth #3 Debunked

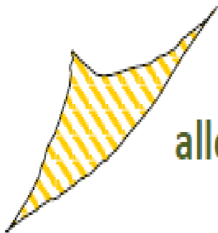
A manufacturer should not take for granted their WCBs

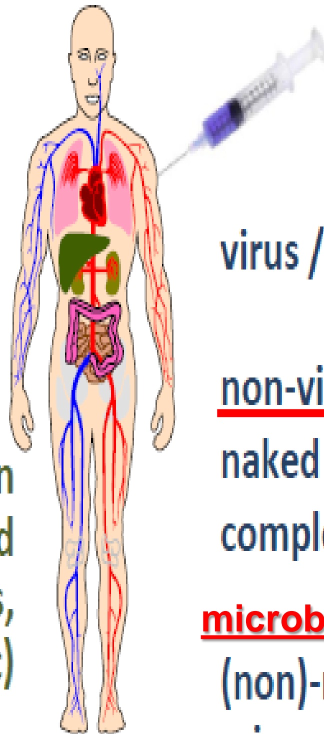
Challenge of regulatory authorities to keep pace with the rapidly developing field of ATMPs

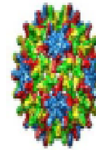
(Numerous source materials: vectors, cells)

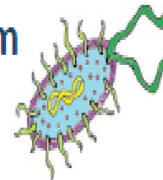












In progress

- ***allogeneic* cells ex vivo gene transfer**
- ***gene editing* ex vivo or in vivo (CRISPR, zinc finger nuclease)**

Comparison to Recombinant Master Cell Banks (rMCBs)

Gene Therapy Source Materials

1) ***Gene therapy source materials have a different level of developmental genetic information to be submitted to initiate clinical development***

2) ***Gene therapy source materials have some of the same patient safety concerns as rMCBs***

1) Gene therapy source materials have a different level of developmental genetic information to be submitted to initiate clinical development

Source, history and generation of the cell substrate

A brief description of the source and generation (flow chart of the successive steps) of the cell substrate, analysis of the expression vector used to genetically modify the cells and incorporated in the parental / host cell used to develop the Master Cell Bank (MCB), and the strategy by which the expression of the relevant gene is promoted and controlled in production should be provided, following the principles of ICH Q5D.

EMA biological IMPD

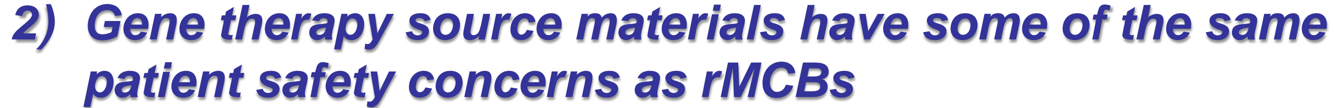
b. Development Genetics

For all vectors, full documentation of the origin where applicable, history and biological characteristics of the parental virus or bacterium should be provided.

All the genetic elements of the GTIMP should be described including those aimed at therapy, delivery, control and production and the rationale for their inclusion should be given. For helper virus, the same level of detail should be provided.

For plasmid DNA, full sequence should be provided.

EMA ATMP IMPD



2) Gene therapy source materials have some of the same patient safety concerns as rMCBs

Similar to rMCBs

- ***Ensure absence of contamination, including sterility, mycoplasma, and in vivo and in vitro testing for adventitious viral agents***
- ***Ensure absence of specific pathogens that may originate from the cell substrate, such as human viruses if the cell line used is of human origin, or pathogens specific to the origin of the production cell line (e.g., murine, non-human primate, avian, insect)***

Unique to Gene Therapy Viruses

- ***Ensure absence of replication competent virus in replication incompetent vectors***

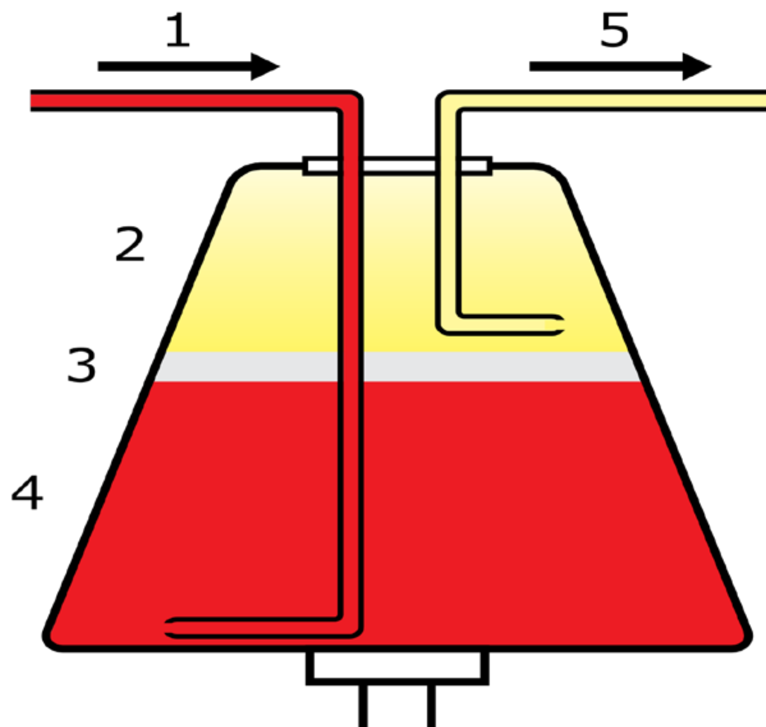
FDA Draft Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) (July 2018)

Source Material for Genetically Engineered Cells

inconsistency of incoming patient cells impacts CQAs

Autologous Therapy

- *Variability in cell type collection (apheresis)*



1 *blood enters centrifuge*
2 *Plasma*
3 *Leukocytes (e.g., T cells)*
4 *Erythrocytes (red blood cells)*
5 *Selected components drawn off*

- ***Obtained not by <u>GMP</u> training of hospital staff***
- ***Obtained by auditing and educating hospital staff; and then the company certifying which clinical sites are acceptable***

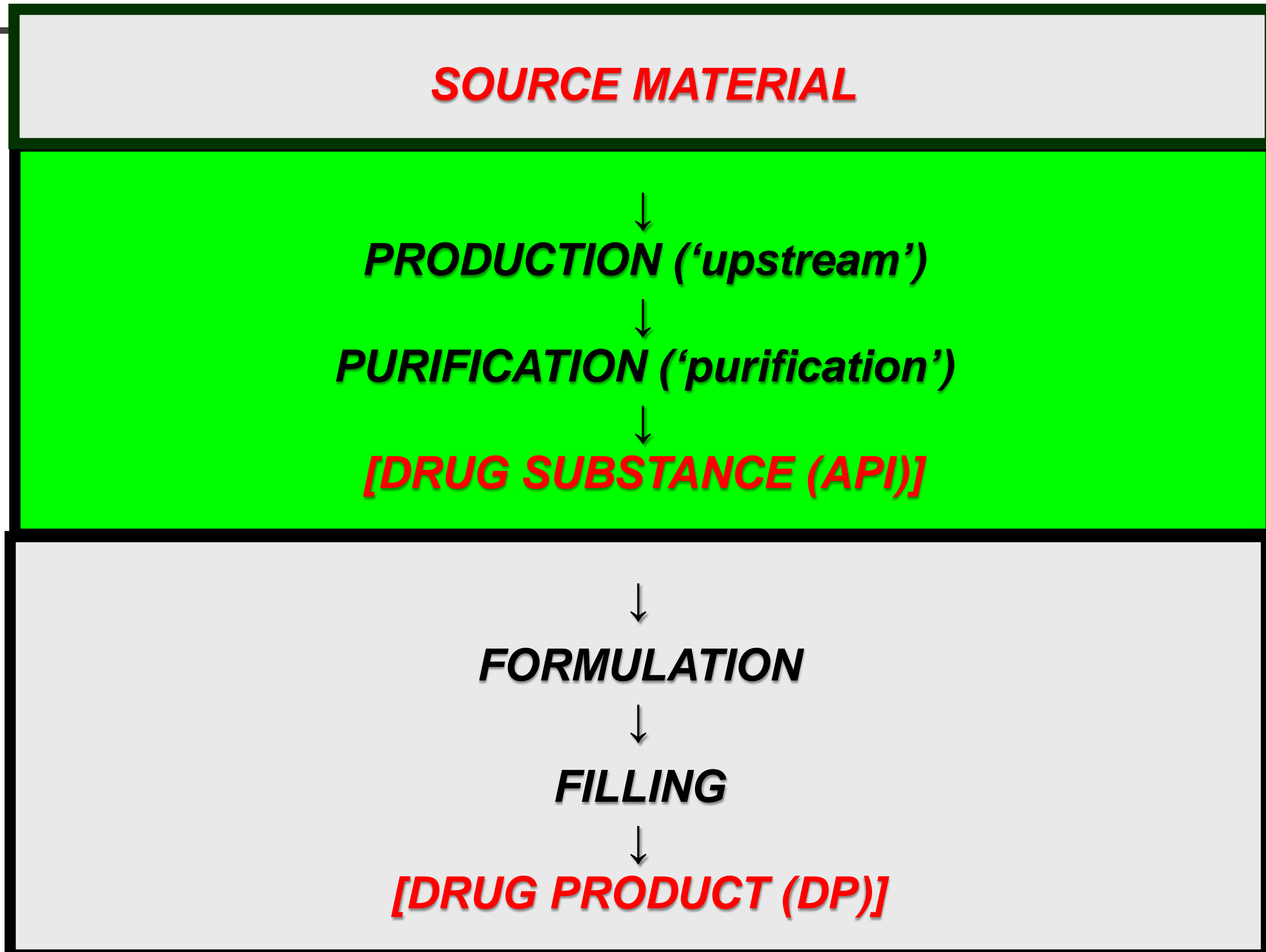
> For Yescarta, Kite/Gilead sends its staff to oversee and educate its supply chain centres. *"We audit the medical facilities, the apheresis and treatment centres, the nurses, the physicians which are going to be using this therapy. We have extensive training programmes as well with them."*
>
> CELL THERAPY MANUFACTURING & GENE THERAPY CONGRESS 2017

- ***Manufacturers take the extra step of further cell processing when received at their site to start with as consistent of the cell type as possible***

Note, Allogeneic Therapy in clinic

Basic Manufacturing Process Flow Diagram

Application of CMC Risk-Managed Control Strategy



Overview of the Manufacture of Biopharmaceutical API Types			
Manufacturing Process	Recombinant Protein/ Monoclonal Antibody	Genetically Engineered Virus	Genetically Engineered Cells
Source Material (contains the genetic elements)	Genetically engineered cell bank	Genetically engineered virus seed bank or plasmid(s) bank	Patient's cells + Genetically engineered virus
↓ Expression	Cell culture (protein induction)	Cell culture (virus transduction or plasmid(s) transfection)	Patient's cells (virus transduction)
↓ Purification	Chromatography to purify *protein* (removal of impurities)	Chromatography to purify *virus* (removal of impurities)	Filtration/washing to purify *cells* (removal of impurities)

'Platform' Monoclonal Antibody Process

Thaw
Working Cell Bank

STEP 1

Seed Maintenance

Seed Culture Expansion in disposable shake flasks and/ or bags

STEP 2

Seed Maintenance

Seed Culture Expansion in fixed stirred tank reactors

N-1 Seed Culture Bioreactor
3,000L WV

STEP 3

Nutrient Feed

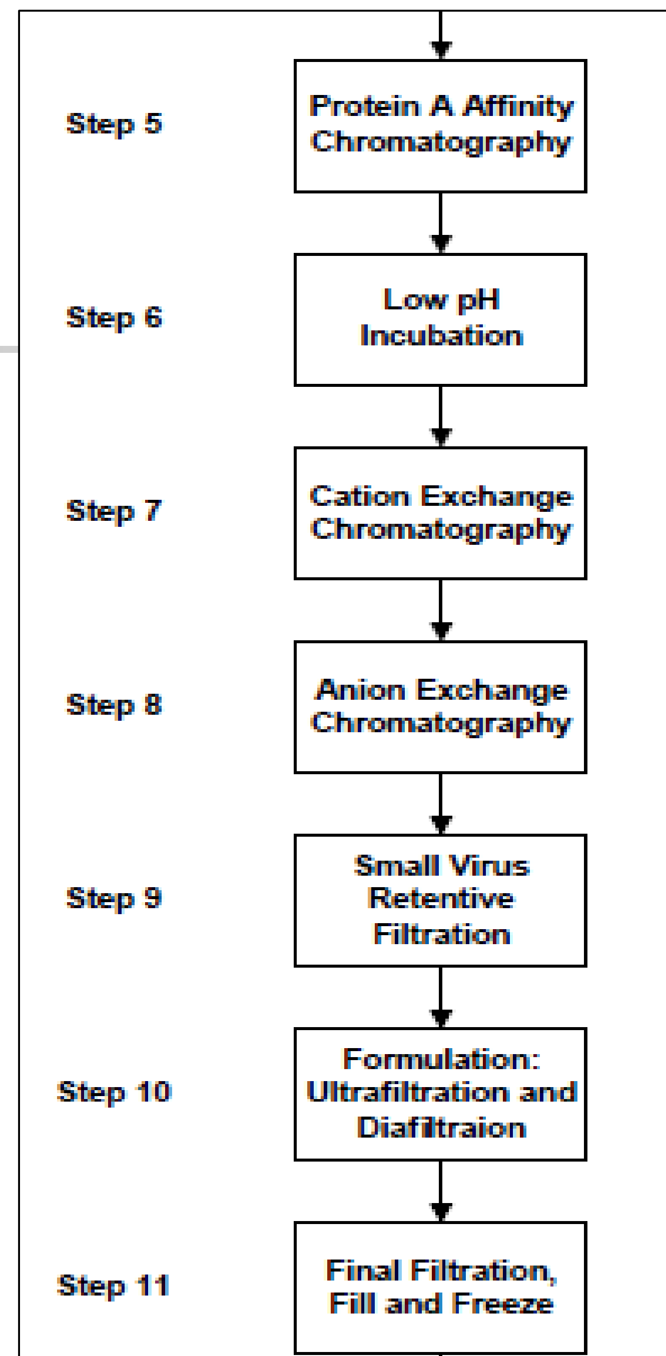
Glucose Feeds

Production Bioreactor
15,000L WV

STEP 4

Harvest
Centrifugation & Depth Filtration

Clarified Bulk

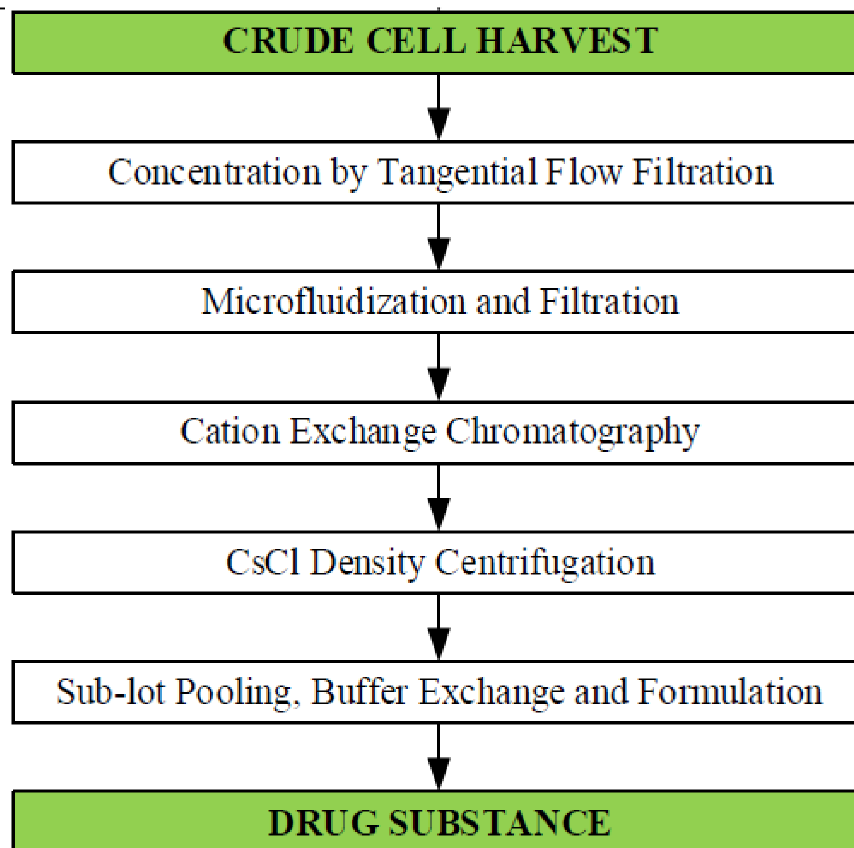


The voretigene neparvovec manufacturing process starts with HEK293 cell culture and expansion, followed by transient transfection with three plasmid constructs. Separate plasmids encode the human RPE65 expression cassette, the AAV2 rep and cap sequences, and the helper virus-derived sequences required for replication of the hRPE65 expression cassette prior to packaging. The transfected cells are harvested, and voretigene neparvovec is purified through multiple processing steps, including final sterile filtration. The Drug Product is formulated as a

Genetically Engineered Virus Process

LUXTURNA™
(voretigene neparvovec)

Briefing Document: October 12, 2017
FDA Advisory Committee Meeting



Many Choices for the Expression System

- Expression systems for producing recombinant proteins/mAbs

Expression System	Commercial Biopharmaceuticals
Bacterial cells	E. coli (>80)
Yeast cells	S. cerevisiae, P. pastoris
Insect cells	S. frugiperda, T. ni, caterpillar
Plant cells	carrot root
Mammalian cells	NSO, CHO (>60), HEK293
Transgenic animals	goat, rabbit, chicken
Transgenic plants	-

- Expression systems for producing genetically engineered viruses

Continuous Cell Lines Used in Virus Production	Commercial Genetically Engineered Virus
VERO (African green monkey)	Imlygic (talimogene laherparepvec) HSV-1
HEK293 (human embryonic kidney)	Luxturna (voretigene neparvovec-rzyl) adeno-associated virus
Sf (Spodoptera frugiperda, fall army worm)	Glybera (alipogene tiparvovec) adeno-associated virus

Many Choices for Cell Culture Operation

- **Batch Mode** – *bioreactor is operated in a closed system with a fixed culture volume in which the cells grow until maximum cell density depending on medium nutrients, product toxicity, waste product toxicity, and other essential factors are reached*
- **Fed-Batch Mode** – *fresh culture medium is added to the bioreactor in fixed volumes throughout the process thus increasing the volume of the cell culture with time, while neither cells nor medium leave the bioreactor*
- **Perfusion Mode (continuous)** – *fresh culture medium is continuously added to the bioreactor while removing an equivalent amount of medium (with or without cells)*

typical protein yields $\geq$ 3 g/L

Many Choices in Bioreactor Types

In-place stainless steel

Single-use, disposable

In-Place Stainless Steel vs Disposable Single-Use Bioreactors

- ***In-Place Stainless Steel***
  - *Samsung BioLogics (www.Samsungbiologics.com) has concluded that in-place large-scale stainless steel bioreactors are preferred for mammalian expression systems, having installed twenty-two 15,000L bioreactors (over 300,000L of capacity) at its manufacturing site in South Korea*
- ***Disposable Single-Use***
  - *WuXi Biologics (www.Wuxibiologics.com) has concluded that single-use bioreactors are preferred for mammalian expression systems, planning on installing over 200,000 L of capacity at its manufacturing site in China*

Where Single-Use Bioreactors are Overwhelmingly Employed

small scale clinical manufacturing
autologous cellular and gene therapy

Need more recombinant protein or monoclonal antibody – scale up!

300,000L of biomanufacturing capacity (20 x 15,000 L)

Need more patient cell batches – scale out!

- Multiple suites and workstations with dedicated equipment
- Off the shelf, bench top equipment
- BSC for aseptic manipulations
- Modular approach
- "Scale out" opposed to "Scale up"

Kite Pharma

Innovative concepts: Bioreactor-in-a-Briefcase!

A future possibility (cell-free biopharmaceutical protein manufacturing)

'Welcome to Betty Crocker bioprocessing'

The portable tech relies on a cell-free expression platform from Thermo Fisher; it lyophilises the contents of a cell, minus the nucleus. "*It's incredible,*" said Rao, "*the entire [raw materials] are freeze-dried powder: welcome to the Betty Crocker world of bioprocessing. Within a few hours you are expressing a high quality protein.*"

These powder kits allow rapid expression of about 500 micrograms of protein per millilitre. "*Imagine no need for cold chain – you can produce on-site and administer to the patient [immediately].*"

UMBC's students even simulated conditions where soldiers use their own body heat to trigger protein production.

The team successfully experimented with human-EPO (erythropoietin), CHO (Chinese Hamster Ovary)-human EPO, and streptokinase "*across three bioreactors. One-and-a-half hours and you're done.*"

The project – a collaboration between Thermo Scientific, UMBC, Ohio State University, Pfizer, FDA, Latham BioPharm Group, Artisan, Dupont, Fluorometric, GE, Genentech, Grace, Merck & Co., and Sartorius-Stedim – was prompted by a $7.9m grant from DARPA, the US Defense Advanced Research Projects Agency.

Recombinant Proteins and Monoclonal Antibodies

The cost of manufacturing biologics has fallen dramatically over the past three decades.

In the early years, the cost of producing biopharmaceuticals in a "legacy" plant could hit $1,000 per gram.

Advances in technology reduced that expense in 1995-2005 to a $100-$500 per gram.

Manufacturers have realized even more savings over the past decade, with the cost now ranging from $50-$100 per gram.

To succeed in the future amid growing competition and pricing pressures, manufacturers will have to get those costs into the $5-$10 range while maintaining or enhancing the level of product quality.

Manufacturing Strategy for Diverse Biologic Pipelines of the Future, Tuft Center for Study of Drug Development, 2017

**The manufacturing process (‘upstream’ and ‘downstream’)
must be adequately and appropriately controlled
to consistently yield a biopharmaceutical API
of acceptable potency, purity, quality and patient safety**

3 Major CMC Regulatory Compliance Issues of Biopharmaceutical API Manufacturing

1) Genetic stability during the cell culture production process

Need to confirm that there is no impact on the quality of the produced product throughout the entire cell culture manufacturing process –

from the beginning (source material) to the end (harvest) of the batch

Genetic Instability Can Occur With All Living Systems

Don't assume 100% genetic fidelity (DNA → RNA → Protein) – __Prove it!__

Membrane

Nucleus

ER

Golgi

Trisulfides...

Plasmid

Genomic DNA

mRNA

pre-mRNA

Protein Synthesis

Processing & Secretion

Mutation in plasmid DNA

Mutation in genomic DNA

Aberrant splicing, Transcription errors

Mistranslation (misreading of codon, mischarged tRNA)

Misincorporations

C-terminal lysine cleavage

Proteolytic clipping

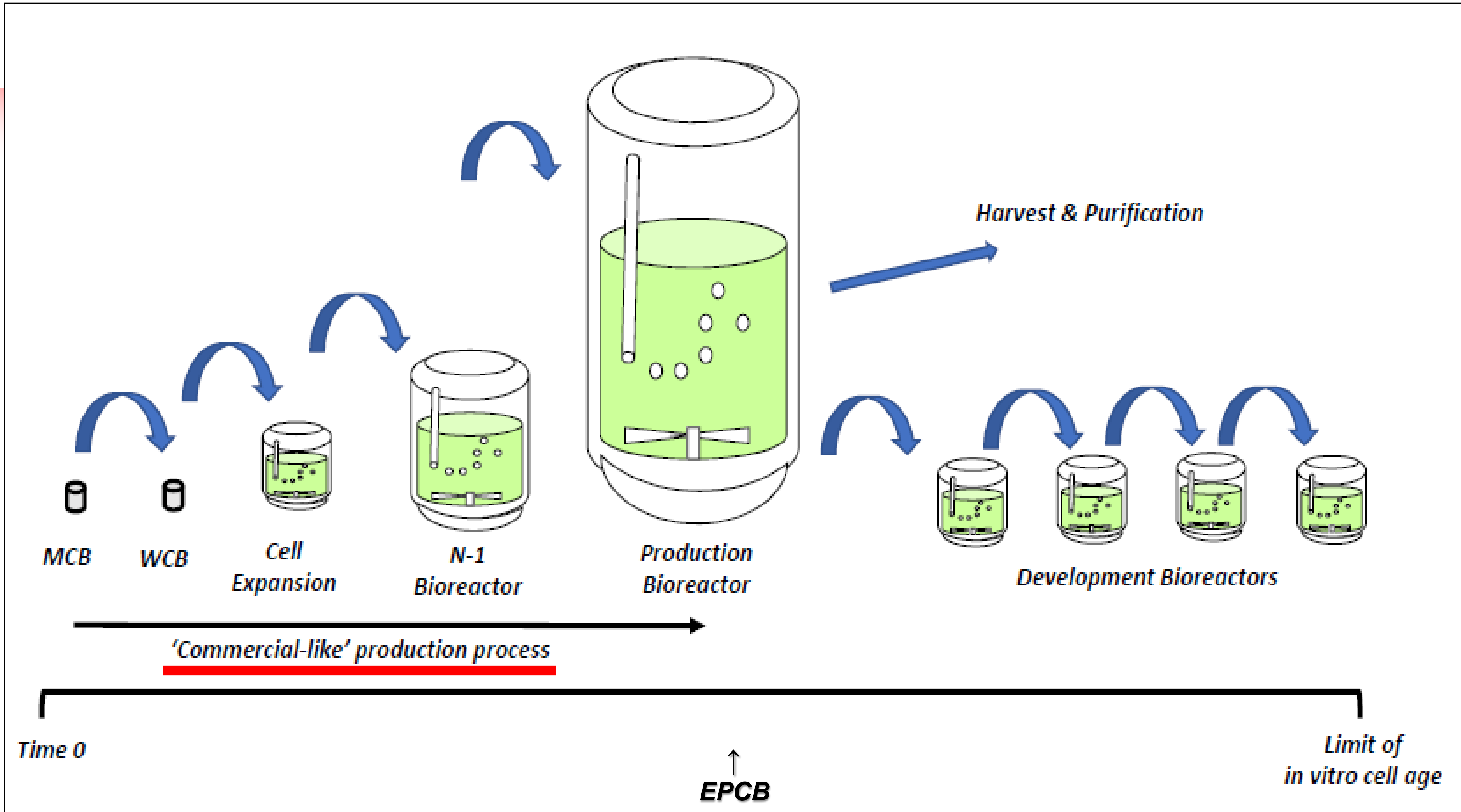
ICH Q5D/Q5A recommendations for genetic stability evaluation

- Perform once for each defined cell culture process
- Test minimally at two time points during production
  - Once at a minimal number of passages
  - Once at the 'limit of in vitro age or beyond'
  - Typical: MCB → WCB → Production End (Harvest) → Extended Culturing
- Determine if there are any genetic or expressed product changes over time – if so, assess the quality impact of the changes
- Test also for latent virus induction (if insect, animal, or human cell line used)

For clinical development → to EPCB

For market approval → to 'at limit'

Traditional & Expected approach to genetic stability determination



Calculation from MCB to 'At Limit':
population doubling, elapsed time, passage number

No regulatory guidance on how long to passage in development

Non-traditional approach to genetic stability determination

(expect regulatory authority hesitancy)

Genentech Perjeta mAb FDA Market Approval Letter June 2012

11. Conduct a study using end of production cells from commercial scale manufacturing that tests for *in vivo* adventitious viruses and genetic consistency. Submit the Final Report as a PAS.

The timetable you submitted on June 1, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	08/2012
Study Completion:	12/2012
Final Report Submission:	02/2013

Rationale for PMC:

The data in the submission for this testing was performed using cells from reduced scale models. Because of concerns regarding the models not being representative of the commercial process, it was determined that this testing would need to be done on cells from the commercial scale process.

Expect regulatory authority questioning of the genetic stability results presented in your submission!

3 Case Examples

- ***Monoclonal antibody produced by Sp2/0 murine cells***
  - ***Significant reduction in copy number (impacted productivity but no impact on product quality)***
- ***Monoclonal antibody produced by CHO cells***
  - ***Reduction in copy number (no impact on productivity or product quality)***
- ***Recombinant protein produced by CHO cells***
  - ***Chromosomal translocation of gene of interest (no impact on productivity or product quality)***

Copy number loss – productivity impacted, but not product quality

Sp2/0 murine cells

Cells at the limit of *in vitro* cell age were characterised from the EPCB and acceptable testing results for the EPCB are provided. Retrovirus particles have been identified, as expected for this cell line. Genetic stability testing for the EPCB compared with the MCB indicated a significant reduction in gene copy number, but although this affects productivity, the quality of CT-P13 from the EPCB was shown to be acceptable. Evaluation using a scale-down model showed similar growth profiles from the MCB to the EPCB, but clear differences in the cumulative product titre were demonstrated. Product quality was

Inflectra MAb (Infliximab Biosimilar) EPAR Hospira 2013

Copy number loss – no impact on productivity or product quality

Determination of the transgene copy number showed 6 copies per cell for light chain and 2 - 3 copies per cell for heavy chain (MCB and WCB), with a slightly lower copy number for the day 19 extended culture samples (5 copies for light chain and 2 copies for heavy chain). While these results might indicate some instability over extended production, no reduction in productivity was detected up to 10 days in the production bioreactor. Differences observed in the SDS-PAGE band pattern at the expected molecular mass for IgG under non-reducing conditions, particularly after 45 passages for the MCB, have been explained. Genetic stability of the WCB and EPCs at mRNA level (in comparison to the MCB) for the intended period of use was confirmed. The potential impact of different copy numbers for light and heavy chain on product quality has been discussed; although there are twice as many gene copies for the light chain in the production cell line, if excess light chain fragments were present these would be removed during the purification process. This is confirmed by the level of low-molecular weight species (LMWS) detected in GMP production runs.

CHO cells

Qarziba (dinutuximab beta) EPAR Apeiron Biologics AG 2017

Chromosomal translocation of gene of interest (GOI) in CHO
Gene relocation – no impact on product quality or productivity

Merck Serono SA

ABSTRACT: During the validation of an additional working cell bank derived from a validated master cell bank to support the commercial production continuum of a recombinant protein, we observed an unexpected chromosomal location of the gene of interest in some end-of-production cells. This event—identified by fluorescence in situ hybridization and multicolour chromosome painting as a reciprocal translocation involving a chromosome region containing the gene of interest with its integral coding and flanking sequences—was unique, occurred probably during or prior to multicolour chromosome painting establishment, and was transmitted to the descending generations. Cells bearing the translocation had a transient and process-independent selective advantage, which did not affect process performance and product quality. However, this first report of a translocation affecting the gene of interest location in Chinese Hamster Ovary cells used for producing a biotherapeutic indicates the importance of the demonstration of the integrity of the gene of interest in end-of-production cells.

Reciprocal Translocation Observed in End-of-Production Cells of a Commercial CHO-Based Process

PDA J Pharm Sci and Tech **2015**, *69* 540-552

1) *Genetic stability during the cell culture production process*

2) Importance, but limitations, of API scaled-down process studies

Small-scale modeling studies are used extensively for biologics

1) ***Number of Experiments Needed**: the more complex the manufacturing process the greater the number of process parameters that need to be studied (using AMBR miniature bioreactors and DOE)*

2) ***Cost Savings**: expensive at full-scale to run a biologic process or to endanger an expensive GMP process step (e.g., spiking excess process-related impurities onto a GMP chromatography column)*

3) ***Not Safe to Carryout at Full-Scale**: in a full-scale biologic manufacturing facility, some studies either cannot be done safely (e.g., worker safety in working with large quantities of live viruses for spiking studies onto columns)*

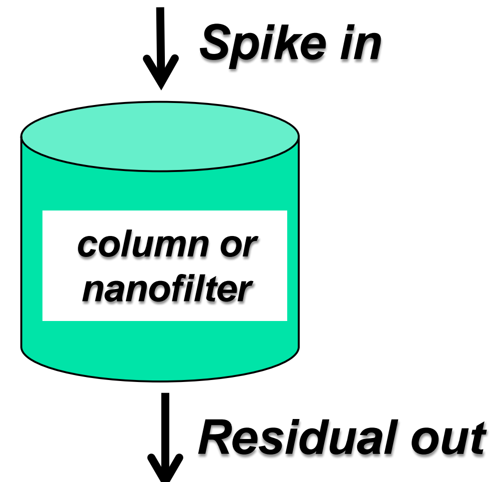
Scaled-down model studies are used across the biopharmaceutical manufacturing process!

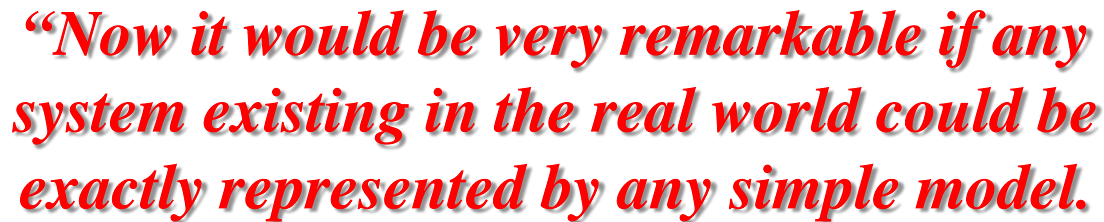
UPSTREAM PROCESS

- *Cell culture media optimization, and identification of critical raw material attributes*
- *Cell culture CPPs (DOE)*
- *Genetic stability (limit in-vitro cell age)*

DOWNSTREAM PROCESS

- *Virus clearance evaluation (chromatography, nanofiltration)*
- *Process-related impurity clearance (host cell DNA and protein, Protein A leachables)*
- *Product-related impurity clearance (oxidation, aggregates)*
- *Process hold times*
- *Chromatographic column resin use life*

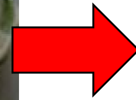


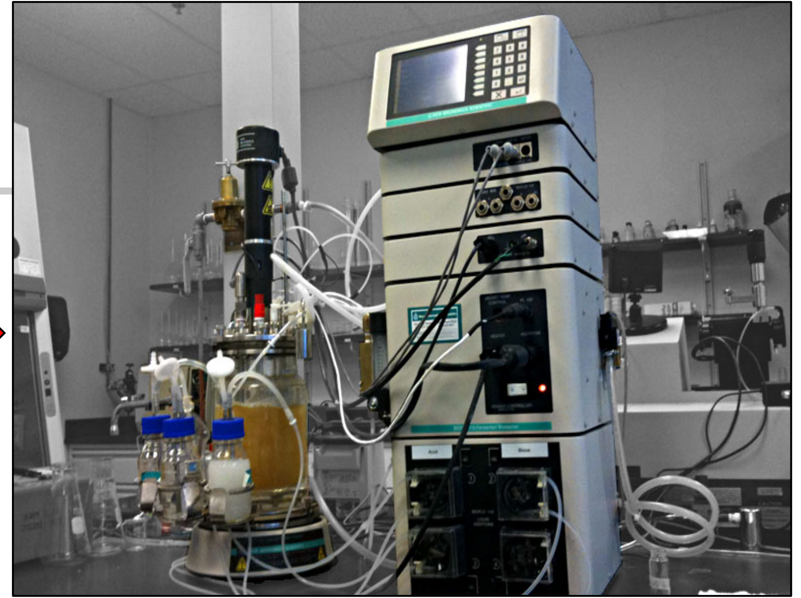


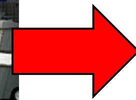
"Now it would be very remarkable if any system existing in the real world could be exactly represented by any simple model. However, cunningly chosen parsimonious models often do provide remarkably useful approximations."

British mathematician and statistician George E P Box

Need to appreciate the limitations of a scaled-down model!







Regulatory authorities understand these limitations

A small scale model must be designed and executed, and ultimately justified, as an appropriate representation of the manufacturing process.

When used, small scale models should be described and their relevance for the commercial scale should be justified, in terms of objective, design, inputs and outputs. When validation studies are highly dependent on the small scale model studies (e.g. design space claimed), it may be necessary to demonstrate that when operating under the same conditions using representative input materials, the outputs resulting from the commercial scale process match those of the small scale model. Any difference in operating conditions, inputs or outputs should be appropriately justified. Depending on

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission

28 April 2016

Regulatory authorities expect documentation of the linkage to the commercial scale manufacturing process

The contribution of data from small-scale studies to the overall validation package will depend upon demonstration that the small-scale model is an appropriate representation of the proposed commercial-scale. Data should be provided demonstrating that the model is scalable and representative of the proposed commercial process. Successful demonstration of the suitability of the small-scale model can enable manufacturers to propose process validation with reduced dependence on testing of commercial-scale batches. Data derived from commercial-scale batches should confirm results obtained from small-scale studies used to generate data in support of process validation. Scientific grounds, or reference to guidelines which do not require or specifically exclude such studies, can be an appropriate justification to conduct certain studies only at small-scale (e.g., viral removal).

ICH Q11

Regulatory authorities will question the design of the scaled-down model

Eli Lilly and Company Trulicity (dulaglutide) **May 30, 2014**

Process characterization studies used to determine the regulatory commitments in the BLA, including the process parameters and in-process controls were inadequate. These studies relied upon the use of small scale models that were not appropriately qualified. For example, the qualifications did not include all CQAs relevant to the unit operations, and the criteria used to evaluate the models were not sufficient. In addition, the process characterization studies themselves were not adequate. For example, all relevant CQAs were not included, and the process parameter ranges studied were, in some cases, too narrow. To address this issue, at the request of the Agency, the sponsor updated sections 3.2.S.2.2, 3.2.S.2.4, 3.2.P.3.3, and 3.2.S.P.3.4 of the BLA with additional regulatory commitments.

FDA Chem Review ***(No delay in market approval)***

1) Genetic stability during the cell culture production process
2) Importance, but limitations, of scaled-down process studies

3) Risk-based control of the biopharmaceutical manufacturing process

Timing for moving from general control to process validation control of the manufacturing process

applies equally to both the API and the DP

Control of the biologic manufacturing process

A learning curve during clinical development!

S.2.4. Control of critical steps and intermediates ***drug substance***

Tests and acceptance criteria for the control of critical steps in the manufacturing process should be provided. It is acknowledged that due to limited data at an early stage of development (phase I/II) complete information may not be available.

P.3.4. Control of critical steps and intermediates ***drug product***

Tests and acceptance criteria for the control of critical steps in the manufacturing process should be provided. It is acknowledged that due to limited data at an early stage of development (phase I/II) complete information may not be available.

Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials

September 2018
EMA/CHMP/BWP/534898/2008 rev. 1

EMA
Process Evaluation

Early Clinical Development Stage

- ***Initially, maybe 1 or 2 manufactured batches to start***
- ***Process validation not expected at this early stage, except for safety***
  - *Media fill hold studies for bioreactor integrity*
  - *Viral clearance safety studies (2 robust steps)*
  - *Media fill hold studies for aseptic processing*

Later Clinical Development Stage

- ***Many more manufactured batches (hopefully)***
- ***Process characterization, QbD***
  - *Identified CQAs and CPPs*

EMA
Process Verification

Biotech:

CTD Module 3 – Process Validation

M4Q(R1)

Sufficient information should be provided on validation and evaluation studies to demonstrate that the manufacturing process (including reprocessing steps) is suitable for its intended purpose and to substantiate selection of critical process controls (operational parameters and in-process tests) and their limits for critical manufacturing steps (e.g., cell culture, harvesting, purification, and modification).

Prospective ***demonstration that the manufacturing process is robust and can yield a consistent product from batch-to-batch***

Biopharmaceutical process validation

Both FDA and EMA have much to say about expectations for the manufacturing process validation

FDA provides the following process validation lists (frequently handed out at pre-BLA meetings with the FDA), associated with confirming product quality microbiology, aseptic processing and sterility

[hot topic list of where they have encountered deficiencies in submitted marketing dossiers]

Drug Substance

3.2.S.2.4 Controls of Critical Steps
3.2.S.2.5 Process Validation/Evaluation
3.2.S.4 Control of Drug Substance

Drug Product

3.2.P.3.5 Process Validation/Evaluation

The CMC Drug Substance section of your BLA (Section 3.2.S) should include the following product quality microbiology information:

- Monitoring of bioburden and endotoxin levels at critical manufacturing steps using qualified bioburden and endotoxin tests. Pre-determined bioburden and endotoxin limits should be provided (3.2.S.2.4).
- Three successful product intermediate hold time validation runs at manufacturing scale. Bioburden and endotoxin levels before and after the maximum allowable hold time should be monitored and bioburden and endotoxin limits provided (3.2.S.2.5). Studies should be performed to determine whether endotoxin recovery is inhibited in material held for the maximum allowable times.
- Column resin and UF/DF membrane sanitization and storage validation data and information (3.2.S.2.5).
- Bioburden and endotoxin data obtained during manufacture of the three conformance lots (3.2.S.2.5).
- Data summaries of shipping validation studies (3.2.S.2.5).
- Drug substance bioburden and endotoxin release specifications. The bioburden limit should be < 1 CFU/10 mL for bulk materials allowed to be stored for extended periods of time at refrigerated temperatures (3.2.S.4).
- Qualification data for bioburden and endotoxin test methods performed for in-process intermediates, buffers, and the drug substance (3.2.S.4).

The CMC Drug Product section of your BLA (Section 3.2.P) should include validation data summaries supporting the aseptic process and sterility assurance. For guidance on the types of data and information that should be submitted, refer to the 1994 "FDA Guidance for Industry, Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products".

- The following study protocols and validation data summaries should be included in Section 3.2.P.3.5:
  - Bacterial retention study for the sterilizing filter.
  - Sterilization and depyrogenation of equipment and components that contact the sterile drug product. The equipment requalification program should be described.
  - In-process microbial controls and hold times. Hold times should be validated at manufacturing scale. Studies should be performed to determine whether endotoxin recovery is inhibited in material held for the maximum allowable times.
  - Isolator decontamination, if applicable.
  - Three successful consecutive media fill runs, including summary environmental monitoring data obtained during the runs. Media fill and environmental monitoring procedures should be described.

Biopharmaceutical process validation

Both FDA and EMA have much to say about expectations for the manufacturing process validation

EMA provides a guideline on process validation for biologic drug substances

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission

28 April 2016

Upstream cell culture process

- ***Bioreactor Conditions**: Evaluation of any critical conditions for the control of expression of the desired product in the production bioreactor is crucial. These activities could include evaluation of specific cell traits or indices (e.g. morphological characteristics, growth characteristics (population doubling level), cell number, viability, biochemical markers, immunological markers, productivity of the desired product, oxygen or glucose consumption rates, ammonia or lactate production rates, process parameters and operating conditions (e.g. time, temperatures, agitation rates, working volumes, media feed, induction of production).*
- ***Harvest**: The conditions utilised to end fermentation/cell culture cycle and initiate harvest should be appropriately defined. Termination criteria should be defined and justified based on relevant information (e.g. yield, maximum generation number or population doubling level, consistency of cell growth, viability, duration and microbial purity and, ultimately, consistency of the quality of the active substance).*

Downstream purification process

- ***Impurity Profile*:** *The capacity of the proposed purification procedures to deliver the desired product and to remove product and process-related impurities (e.g. unwanted variants, HCPs, nucleic acids, media components, viruses and reagents used in the modification of the protein) to acceptable levels should be thoroughly evaluated.*
- ***Viral Clearance*:** *Evaluation of steps where viral clearance is claimed should be performed as described, according to ICH Q5A (R1).*
- ***Chromatography Resin Use Life*:** *Columns should also be evaluated throughout the expected lifetime of the column regarding purification ability (e.g. clearance, peak resolution in separation of isoforms), leaching of ligands (e.g. dye, affinity ligand) and/or chromatographic material (e.g. resin).*
- ***Hold Times*:** *Where process intermediates are held or stored, the impact of the hold times and conditions on the product quality from a structural and microbial point of view should be appropriately evaluated. The evaluation should be conducted as real-time, real-condition studies, usually on commercial scale material.*

What about the ‘3 Run Rule’ for commercial process validation?

‘validation batches’, ‘conformance batches’, ‘PPQ batches’

***3** consecutive manufactured batches of*
drug substance
representative of the commercial scale
and its product quality (i.e., released batches)

***3** consecutive manufactured batches of*
drug product
representative of the commercial scale
and its product quality (i.e., released batches)

What happened to the ‘5 consecutive batches’ previously imposed by EU?

What is the origin of ‘3’?