CMC Regulatory Compliance Strategy For Biopharmaceuticals

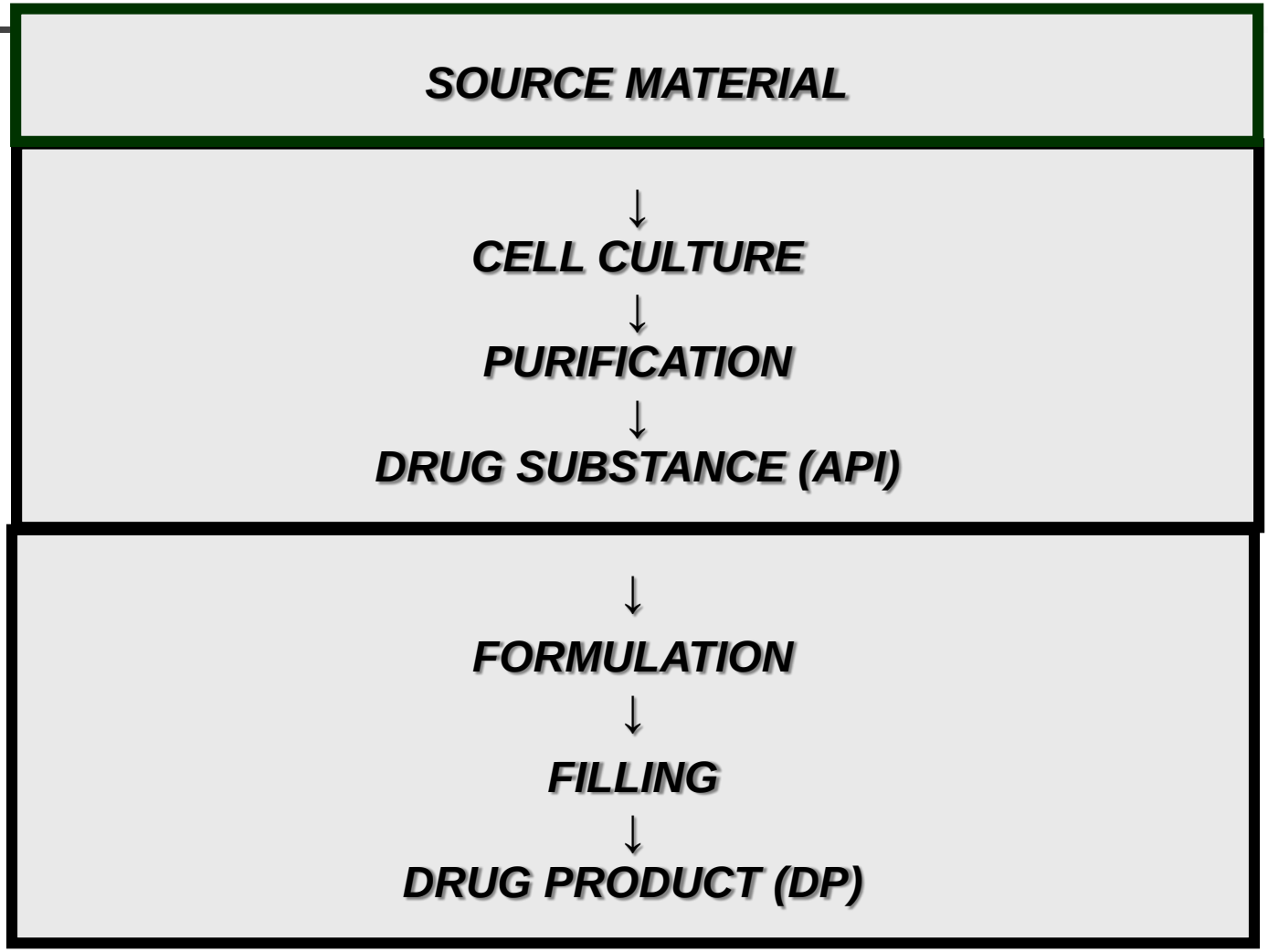
Course Outline

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

- ✓ Walking through the 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 and contrasting the challenges between the protein-based, virus-based and cell-based biopharmaceutical manufacturing processes

Basic Manufacturing Process Flow Diagram

Application of CMC Risk-Managed Control Strategy



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)**

***Biopharmaceutical*: the source material contains the genetic capability of producing the desired biopharmaceutical product**

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

Biopharmaceutical source materials containing genetic capability:

(1) genetically engineered cell banks (for producing recombinant proteins, monoclonal antibodies, recombinant DNA plasmids)

(2) genetically engineered virus banks (viral vector in gene therapy)

(3) recombinant DNA plasmid banks (for transiently producing genetically engineered virus)

(4) genetically engineered bacterial banks (microbial vector in gene therapy)

(5) transgenic banks (for producing recombinant proteins in transgenic plants or animals)

Source material for a biopharmaceutical

Biologic Type	Source Material
Recombinant Proteins & Monoclonal Antibodies	Master Cell Bank (MCB)
Genetically Engineered Viruses for Gene Therapy	Master Virus Bank (MVB) Master Plasmid Construct Bank (MPCB)

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

Assembling the Recombinant Master Cell Bank

(Step 1) Obtaining the basic genetic components

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

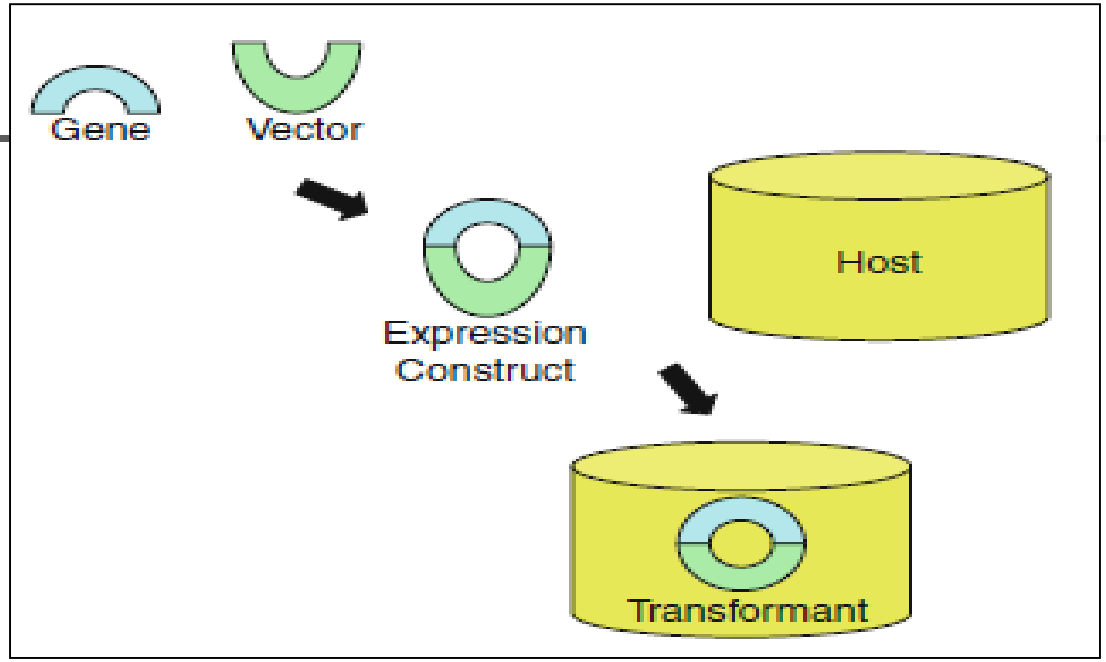
- ***Vector*** *– larger piece of DNA (e.g., plasmid, virus) that contains promoters, enhancers and other genetic pieces to allow the gene to function and survive within a foreign host*

Expression construct *– gene inserted into vector (frequently a plasmid)*

- ***Host*** *– living cell into which the expression construct is to be inserted that enables the gene to function*

Assembling the Recombinant Master Cell Bank

(Step 2) Developmental Genetics



- ***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)*

Transformed Cells

↓

***Cloning* – selection of a single recombinant cell/virus/plasmid 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

Manufacture of the Recombinant Master Cell Bank
(Step 3)

Cell Substrate

↓

Master Cell Bank (MCB)

the expanded cell substrate Is dispensed 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 dispensed 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)***

Expectations of all Master Banks (MCB, MVB, MPCB)

- ***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

Clinical Master Cell Bank is always acceptable for commercialization!

To initiate human clinical studies

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. Information on the generation, qualification and storage of the cell banks is required. The MCB and/or WCB if used should be characterised and results of tests performed should be provided. Clonality of the cell banks should be addressed for mammalian cell lines. The generation and characterisation of the cell banks should be performed in accordance with the principles of ICH Q5D. Cell banks should be characterised for relevant phenotypic and genotypic markers so that the identity, viability, and purity of cells used for the production are ensured. The nucleic acid sequence of the expression cassette including sequence of the coding region should be confirmed prior to the initiation of clinical trials.***

EMA Guideline on the Requirements for Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trials (September 2017)

But, the key focus during human clinical studies is patient safety

the regulatory reviewer will 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

absence of adventitious agents of concern

- **Prions – TSEs**
  - Prevented through raw material control in preparing bank
- **Viruses – animal/human**
  - Extensive viral safety testing of bank; $$$
- **Mycoplasmas**
  - 28 day testing of bank
- **Bacteria/Fungi**
  - Culture purity testing of bank (if bacterial/yeast)
  - Sterility testing of bank (if animal/human)

ICH Q5D

Patient Safety Focus

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

Cell lines do get mixed-up! Especially if handled in R&D

Where was your genetic engineering done?
Purity confirmed by documentation of procedural controls

Patient Safety Focus

identity (characterization) of genetic components

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

ICH Q5B

ICH Q5D

To obtain market approval, a more thorough review of the provided information occurs

- When it is time to consider market approval for the recombinant protein or monoclonal antibody, patient safety continues to remain the primary regulatory evaluation of the MCB
- But at this time, not only is the MCB more thoroughly reviewed from a patient safety perspective, but also the MCB is reviewed to determine if it can truly yield a stable, continuous, homogenous source for future manufacturing
- The detailed information in the filed market application dossier on the developmental genetics, the MCB characterization and its long-term stability are now thoroughly reviewed

I. **Host Cells** – A description of the source, relevant phenotype, and genotype should be provided for the host cell used to construct the biological production system. The results of the characterization of the host cell for phenotypic and genotypic markers, including those that will be monitored for cell stability, purity, and selection should be included.

II. **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. A description of the method(s) used to prepare the gene construct and a restriction enzyme digestion map of the construct should be included. 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.

III. **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. A restriction enzyme digestion map indicating at least those sites used in construction of the vector should be provided. The genetic markers critical for the characterization of the production cells should be indicated.

IV. 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. A restriction enzyme digestion map indicating at least those sites used in construction of the final product construct should be provided.

V. Cloning and Establishment of the Recombinant Cell Lines – Depending on the methods to be utilized to transfer a final gene construct or isolated gene fragments into its host, the mechanism of transfer, copy number, and the physical state of the final construct inside the host cell (i.e. integrated or extrachromosomal), should be provided. In addition, the amplification of the gene construct, if applicable, selection of the recombinant cell clone, and establishment of the seed should be completely described.

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)

Documentation of the developmental genetics is important!

part of the Regulatory Authority safety assessment

It is important to provide supportive documentation which describes the history of the cell substrate that is used in the manufacture of a biotechnological/biological product, as well as any parental cell line from which it was totally or partially derived. Events during the research and development phases of the cell substrate may contribute significantly to assessment of the risks associated with the use of that particular cell substrate for production. The information supplied in this regard is meant to facilitate an overall evaluation which will ensure the quality and safety of the product.

ICH Q5D

"Garbage in, Garbage out!"

What happens upstream (genetic engineering, clone selection process, cell banking) flows downstream!

Surprises are discovered in MCBs

***after* clinical development is completed**

Case Examples of MCB Concerns

- ***Genetic identify of assembled components***
- ***Virus safety***
- ***Proof of clonality***

Discovered MCB concern about identity of genetic components

***after* clinical development is completed**

Recombinant Protein produced by Recombinant Carrot Cells

Elelyso (Taliglucerase Alfa)

According to … ICH Q5B, the purpose of analyzing the expression construct is to establish that the correct coding sequence of the product has been incorporated into the host cell and is maintained during culture to the end of production. 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 about virus safety

***after* clinical development is completed**

Recombinant Protein produced by CHO

Vimizim (Elosulfase Alfa)

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)

Discussion

MCB – proof of clonality

an important concern by the regulatory authorities

***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)*

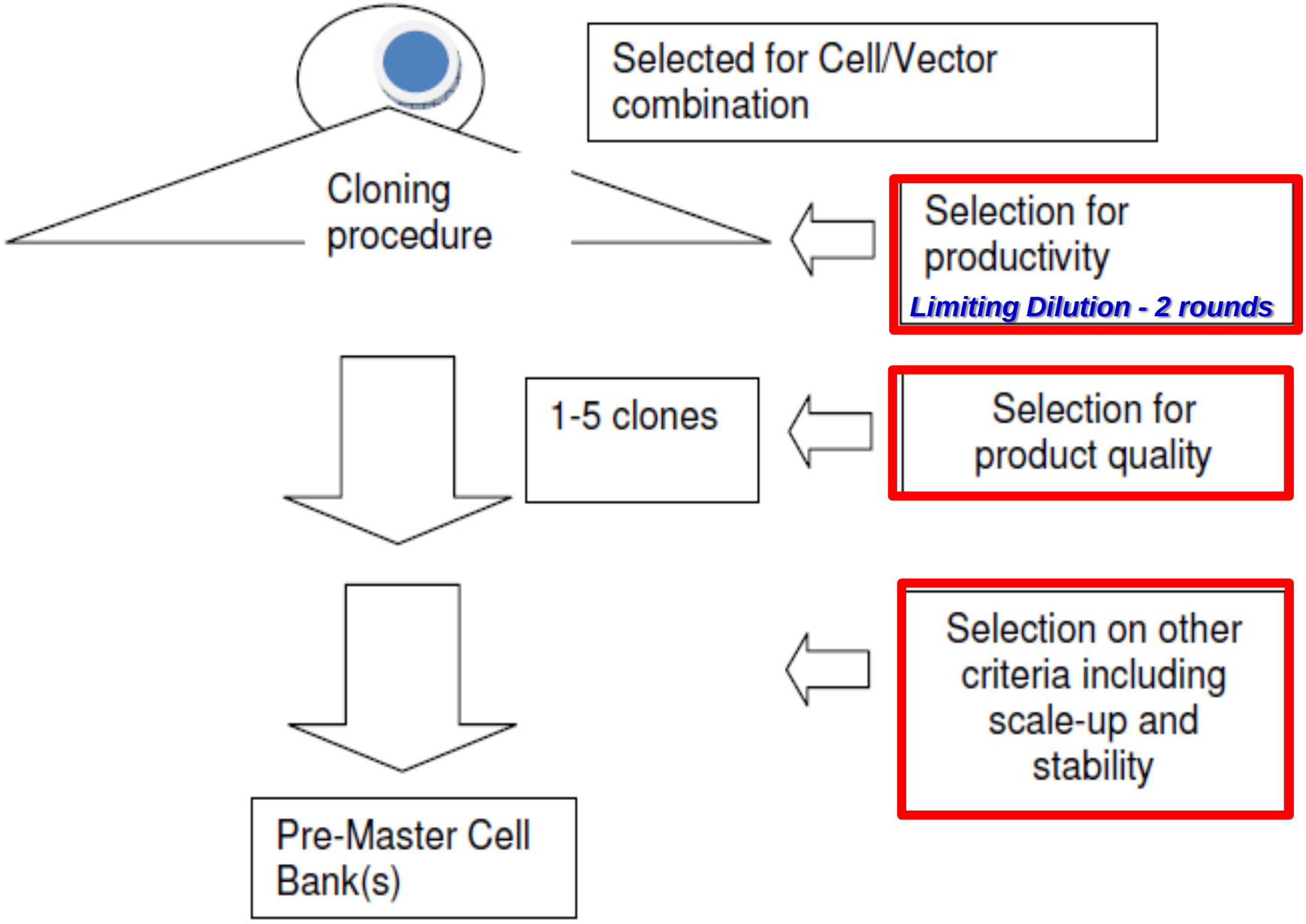
Transformed cells → Cloning → Cell Substrate → MCB

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!


Selected for Cell/Vector combination
Cloning procedure
Selection for productivity
Limiting Dilution - 2 rounds
1-5 clones
Selection for product quality
Selection on other criteria including scale-up and stability
Pre-Master Cell Bank(s)

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

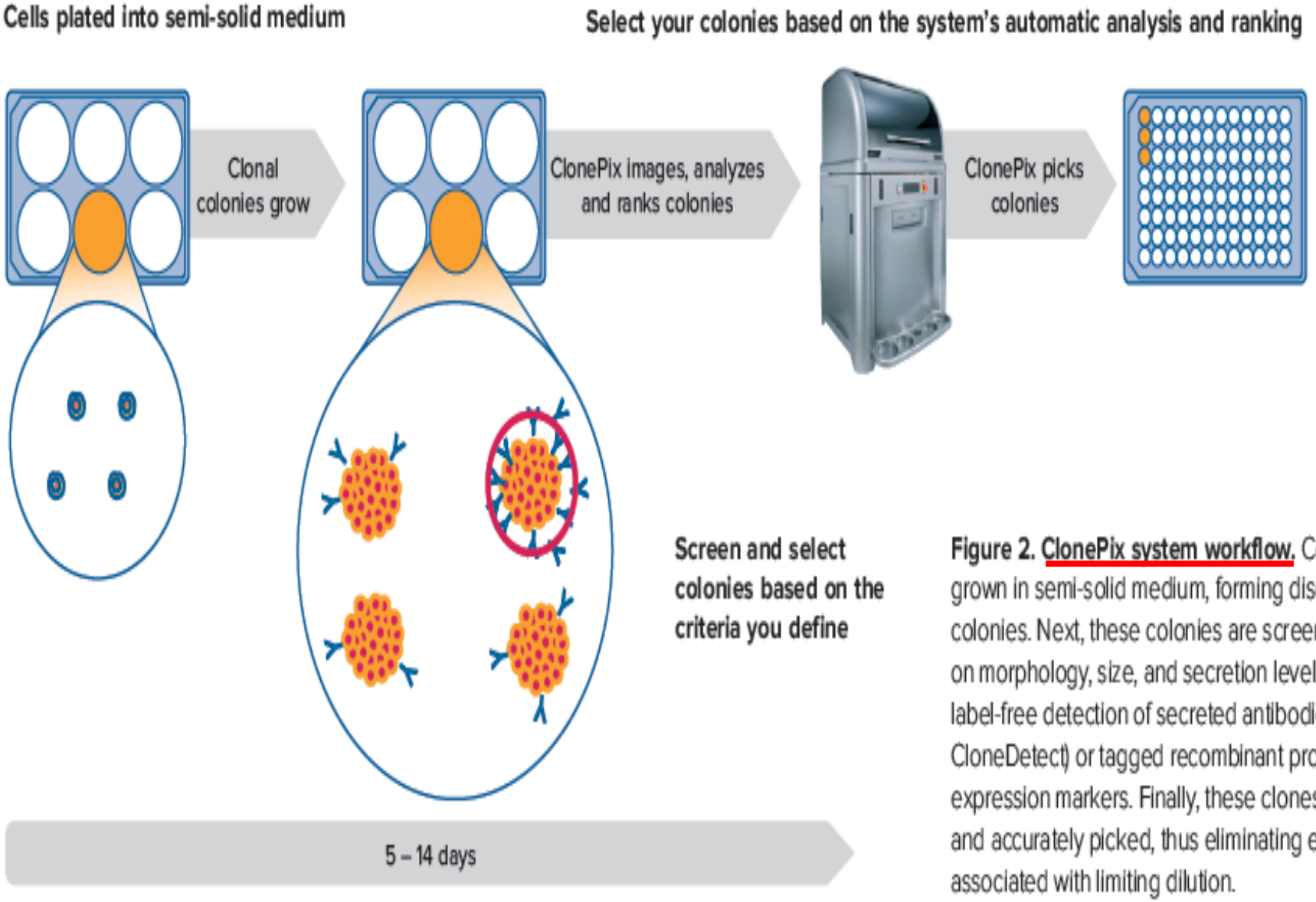
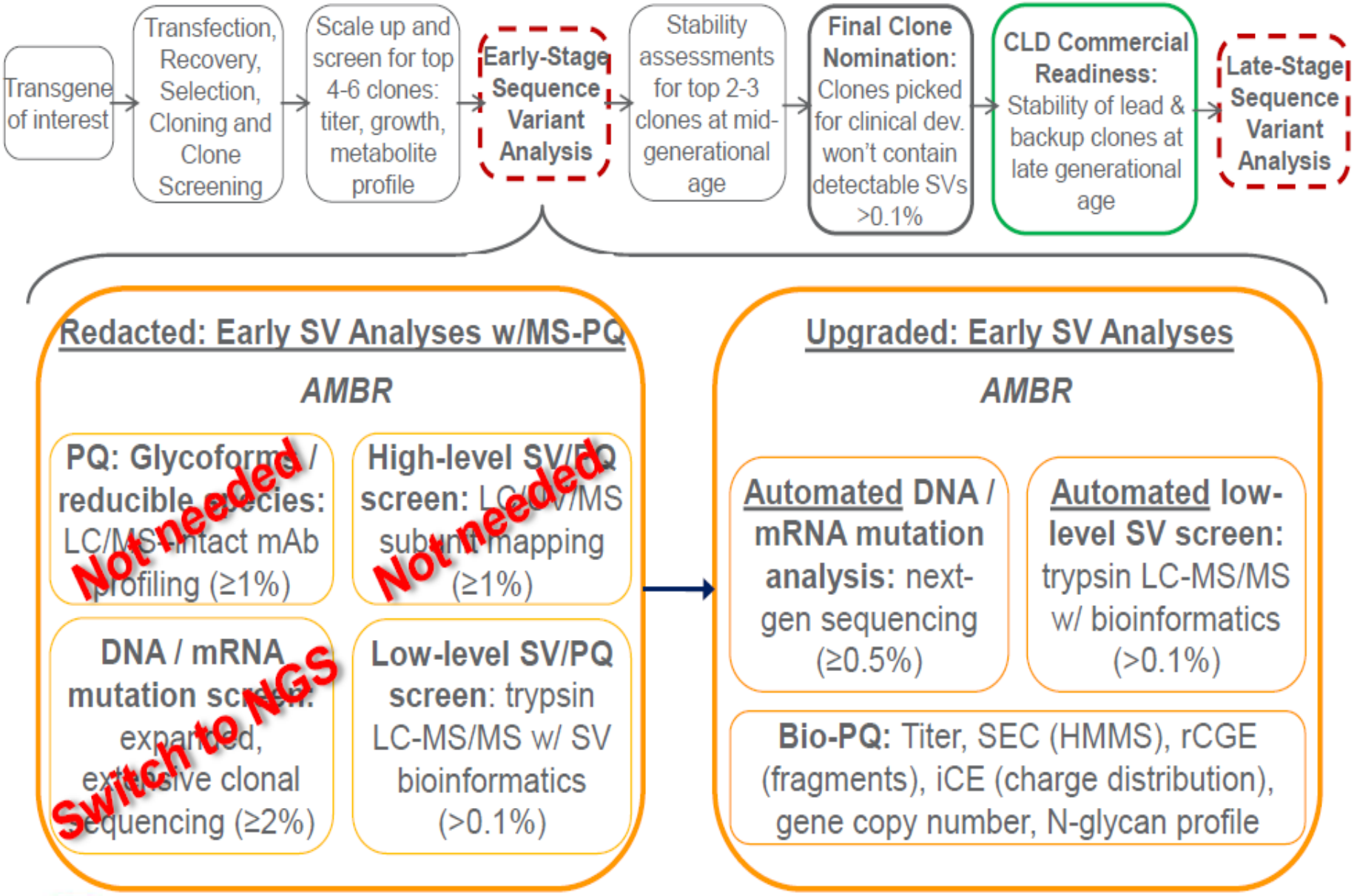


Figure 2. ClonePix system workflow. Cells are grown in semi-solid medium, forming discrete clonal colonies. Next, these colonies are screened based on morphology, size, and secretion level using label-free detection of secreted antibodies (such as CloneDetect) or tagged recombinant proteins and expression 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



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

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

- 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

- 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.

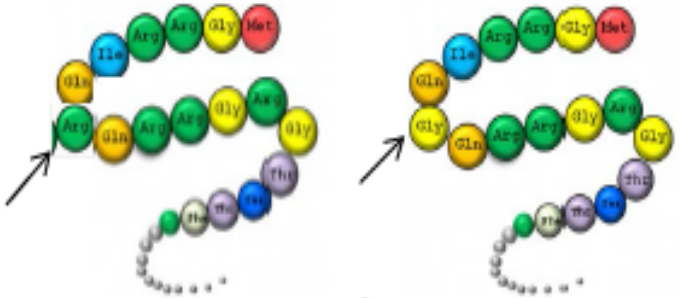
R. Novak, CDER, WCBP 2017

Preparing the CMC section of IMPD for biological/biotechnology derived substances

Monoclonality

Dr. Una Moore
Health Products Regulatory Authority, Ireland

16th April 2014.



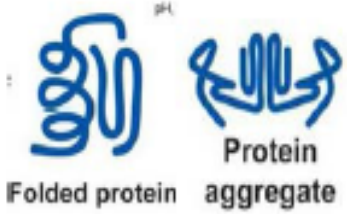
The DS could be a mixture e.g.

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

Regulatory authority options, if concerned about lack of proof of clonality

- ***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

Discovered MCB concern about proof of clonality

***after* clinical development is completed**

Monoclonal Antibody produced by CHO

Crysvita (Burosumab)

The establishment of burosumab MCB includes multiple selection procedures for the cells that produce burosumab with adequate growth profiles. However, a formal cloning procedure was conducted only once . Therefore, there is residual uncertainty for the monoclonality of burosumab MCB.

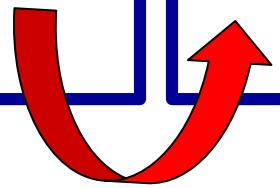
The goal of the study is to demonstrate consistent genetic profiles for the subclones of burosumab MCB to ensure the monoclonality of burosumab MCB. The specifications for burosumab drug substance and drug product are acceptable to ensure adequate quality and safety for the initial marketed product. 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)

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 only 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***

1) Cell bank inventory level

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.

In the case when production does not take place for a long period of time, viability testing on the cell bank used as a source of the production substrate should be performed at an interval described in the marketing application.

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

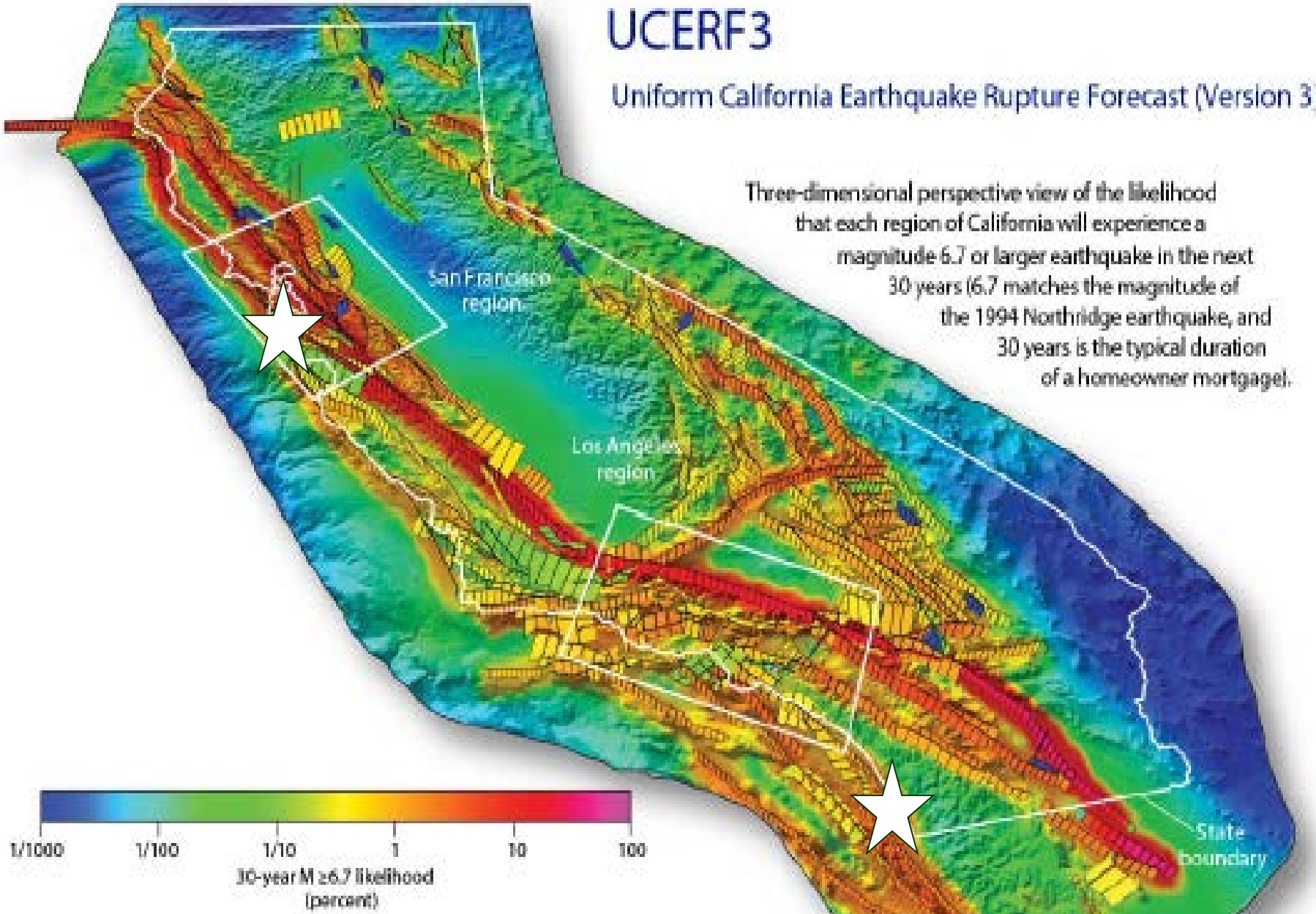
What if the unthinkable happens?

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., Maria – Puerto Rico 2017)



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

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

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

There are justifiable reasons to replace a MCB during clinical development!

GMP Compliance Reasons

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

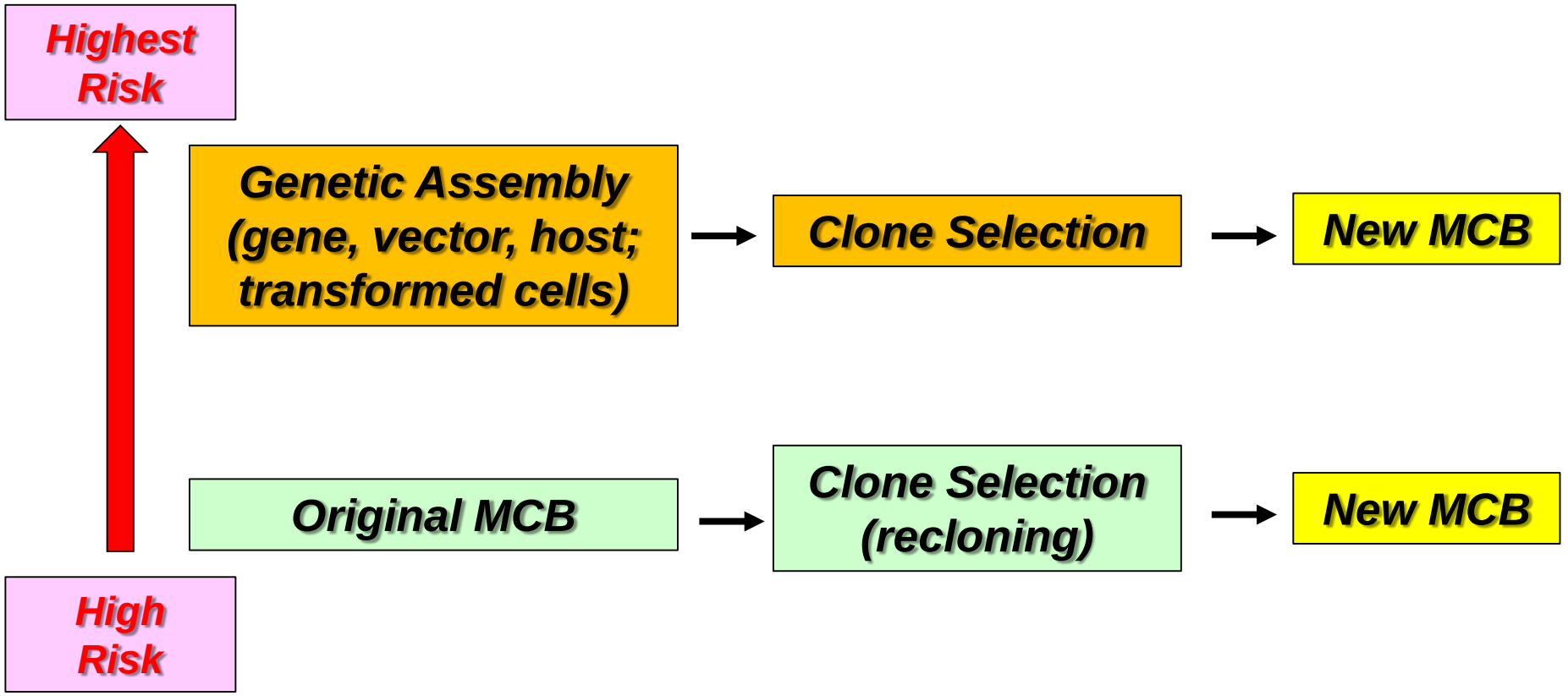
Quality Reasons

- *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 manufacturing process changes carry risk!



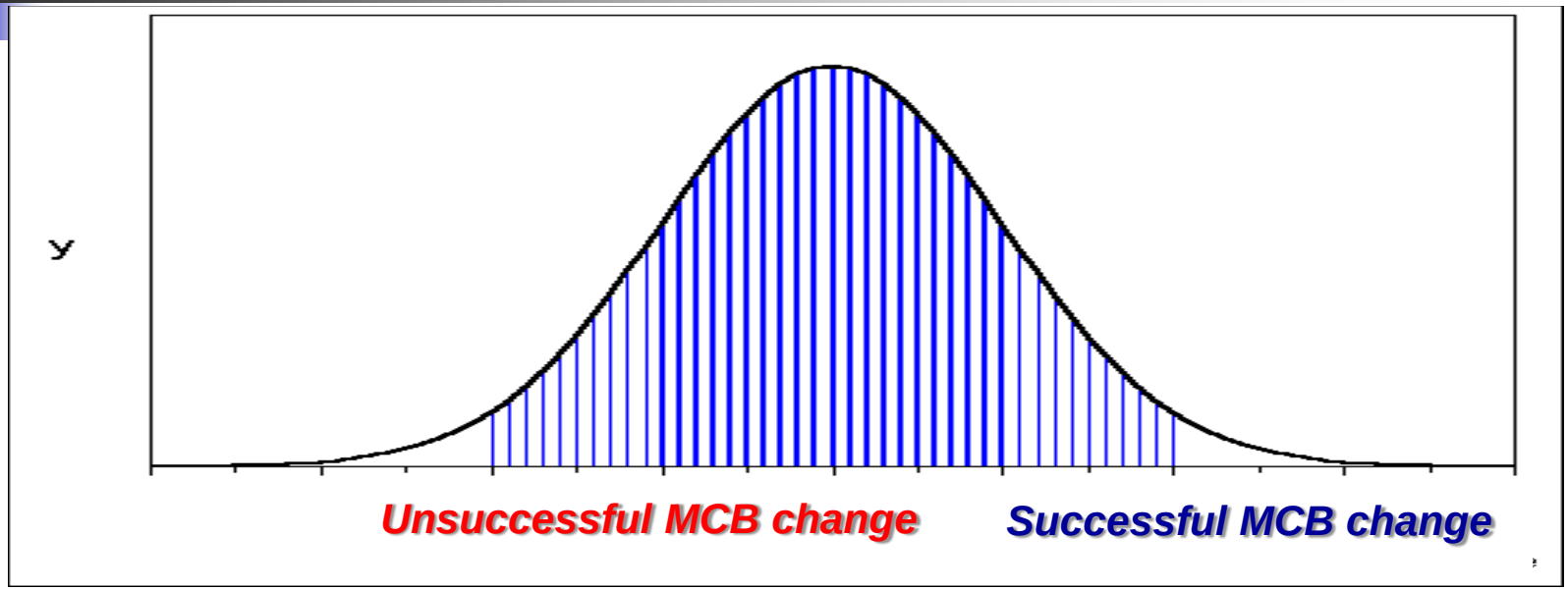
MCB exchange out requires regulatory authority prior approval!

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!

Regulatory authorities are aware of the risks associated with the introduction of new WCBs manufactured from a MCB

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 2017)

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)

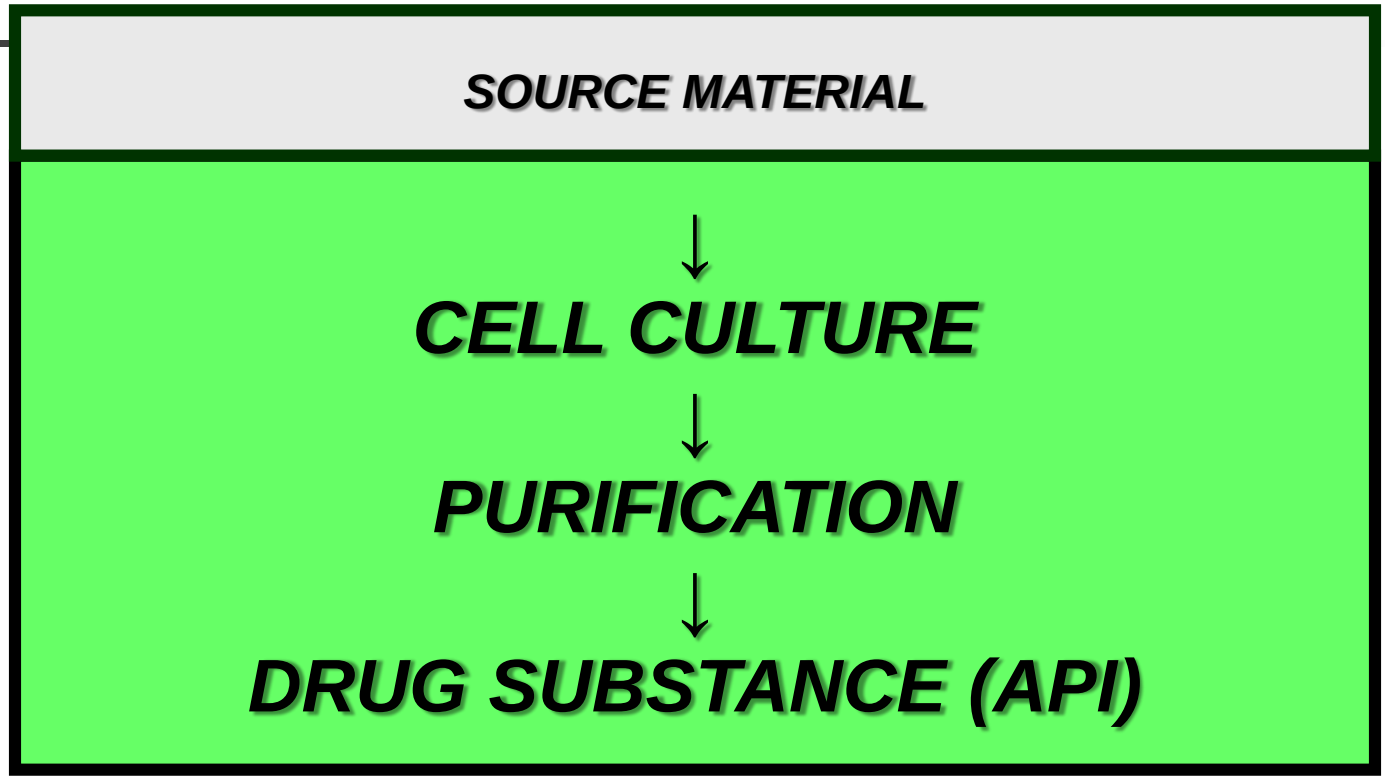
FDA Drugs – Search Drugs@FDA: FDA Approved Drug Products: Perjeta (Pertuzumab) – Approval History, Letters, Reviews and Related Documents – Market Approval Letter (June 08, 2012)

Myth #3 Debunked

A manufacturer should not take for granted their WCBs

Basic Manufacturing Process Flow Diagram

Application of CMC Risk-Managed Control Strategy



Choices of 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
Plant cells	carrot root
Mammalian cells	CHO (>50) BHK, murine myeloma/hybridoma
Transgenic animals	goat, rabbit, chicken
Transgenic plants	-

- Expression systems for producing genetically engineered viruses

Expression System	Commercial Biopharmaceuticals
Mammalian cells	VERO (African green monkey) HEK293 (human embryonic kidney)

Choices of 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 ≥ 3 g/L

Choices of Bioreactors

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***

Major Acceptance of Single-Use Bioreactors

small scale clinical manufacturing
autologous cellular and gene therapy

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.*"

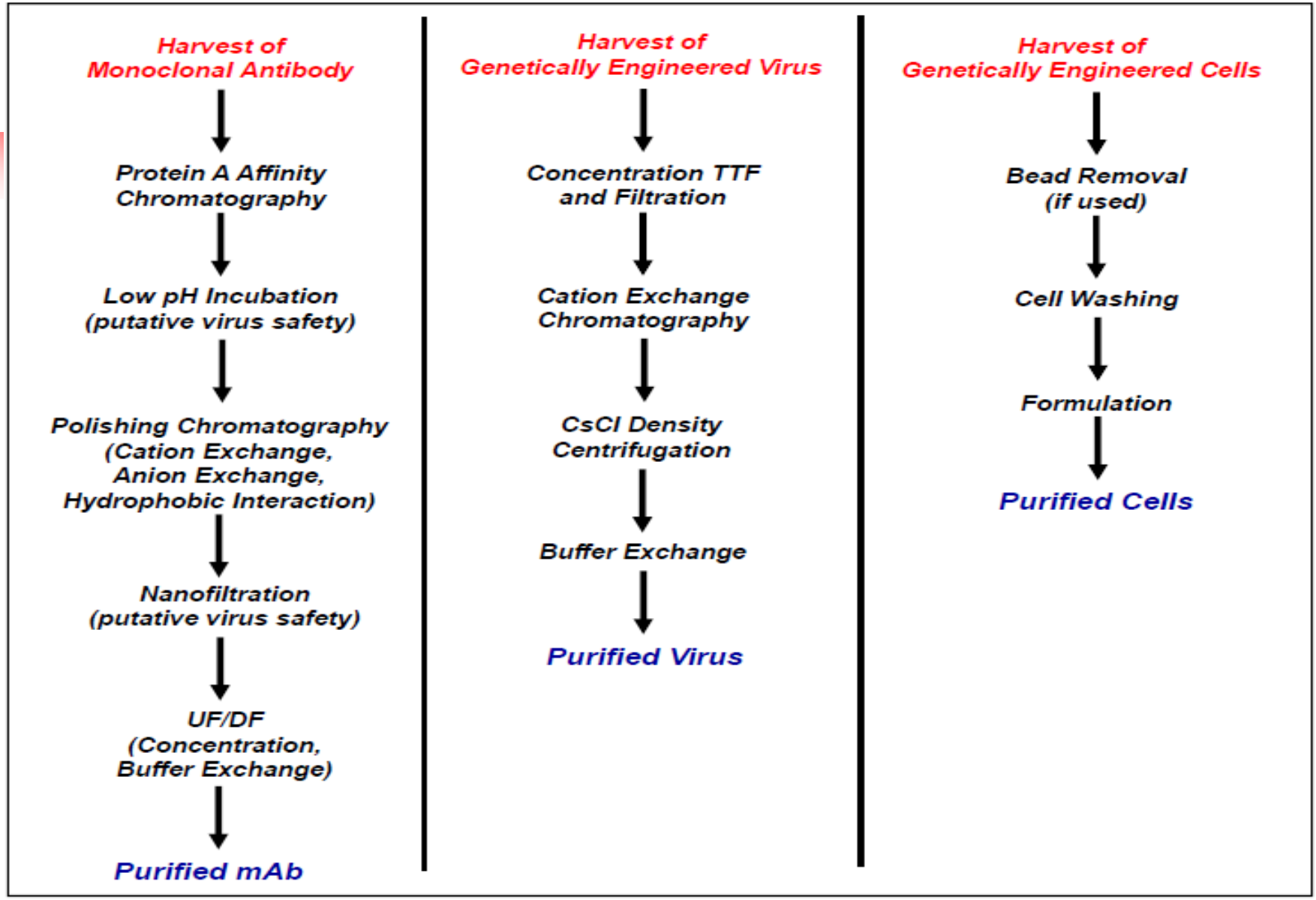
These powder kits allow rapid expression of about 500 micrograms of protein per millilitre. "*Imagine no need for cold chain – you can produce on-site and administer to the patient [immediately].*"

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.

"Downstream" purification process for biopharmaceutical APIs



similar, but not identical, chromatography

no chromatography

The Challenge Ahead!

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 per-gram range of $100-$500.

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

Regardless of the API manufacturing process employed or its manufacturing scale, the regulatory authorities have one major concern!

The manufacturing process must be adequately and appropriately controlled to consistently yield a biopharmaceutical API of acceptable quality and patient safety

This concern extends from the 'upstream' production process steps to the 'downstream' purification process steps

3 Major CMC Regulatory Compliance Issues of API Manufacturing

3 Major CMC Regulatory Compliance Issues of API Manufacturing

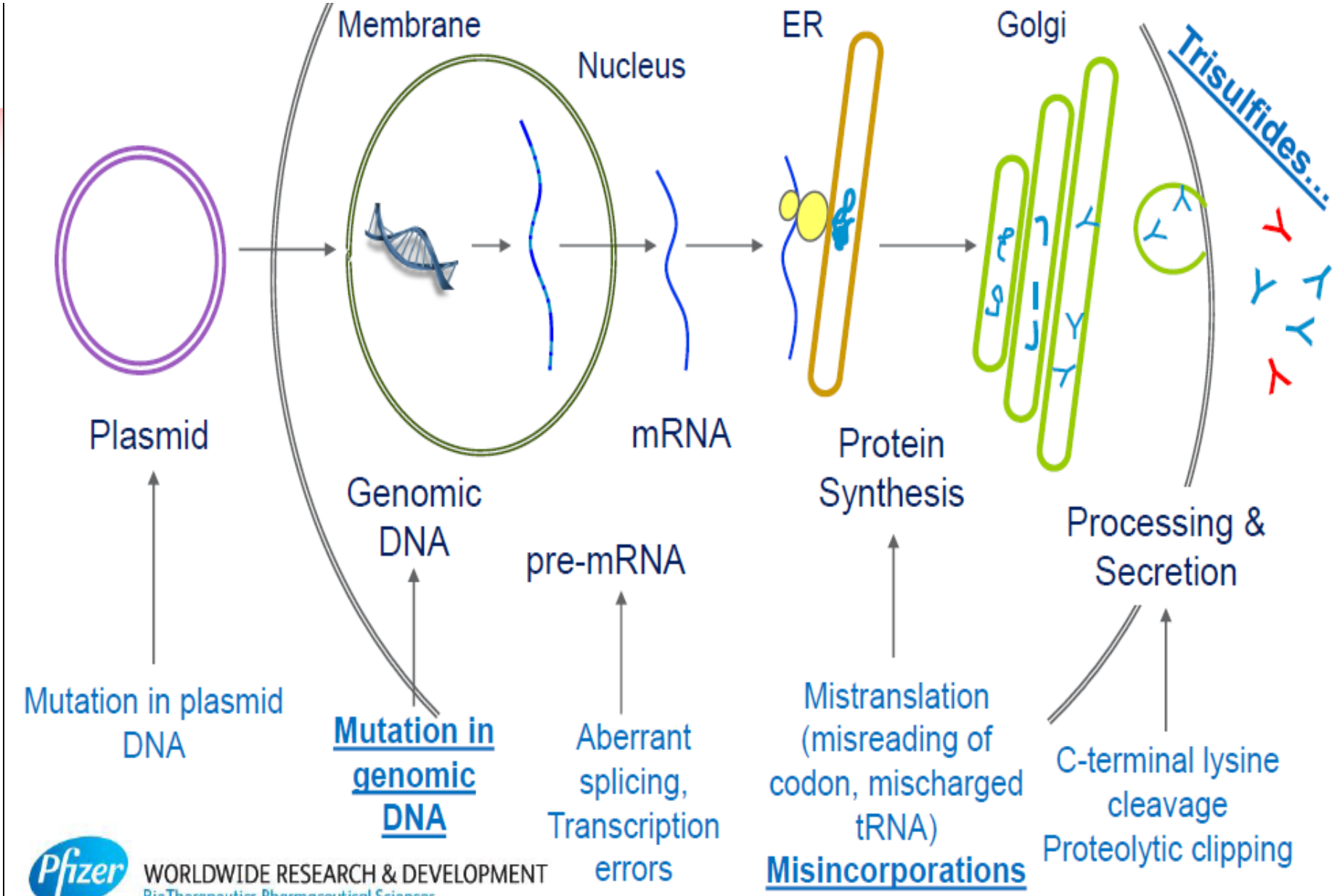
1) Genetic stability during the cell culture production process

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) – <u>Prove it!</u>

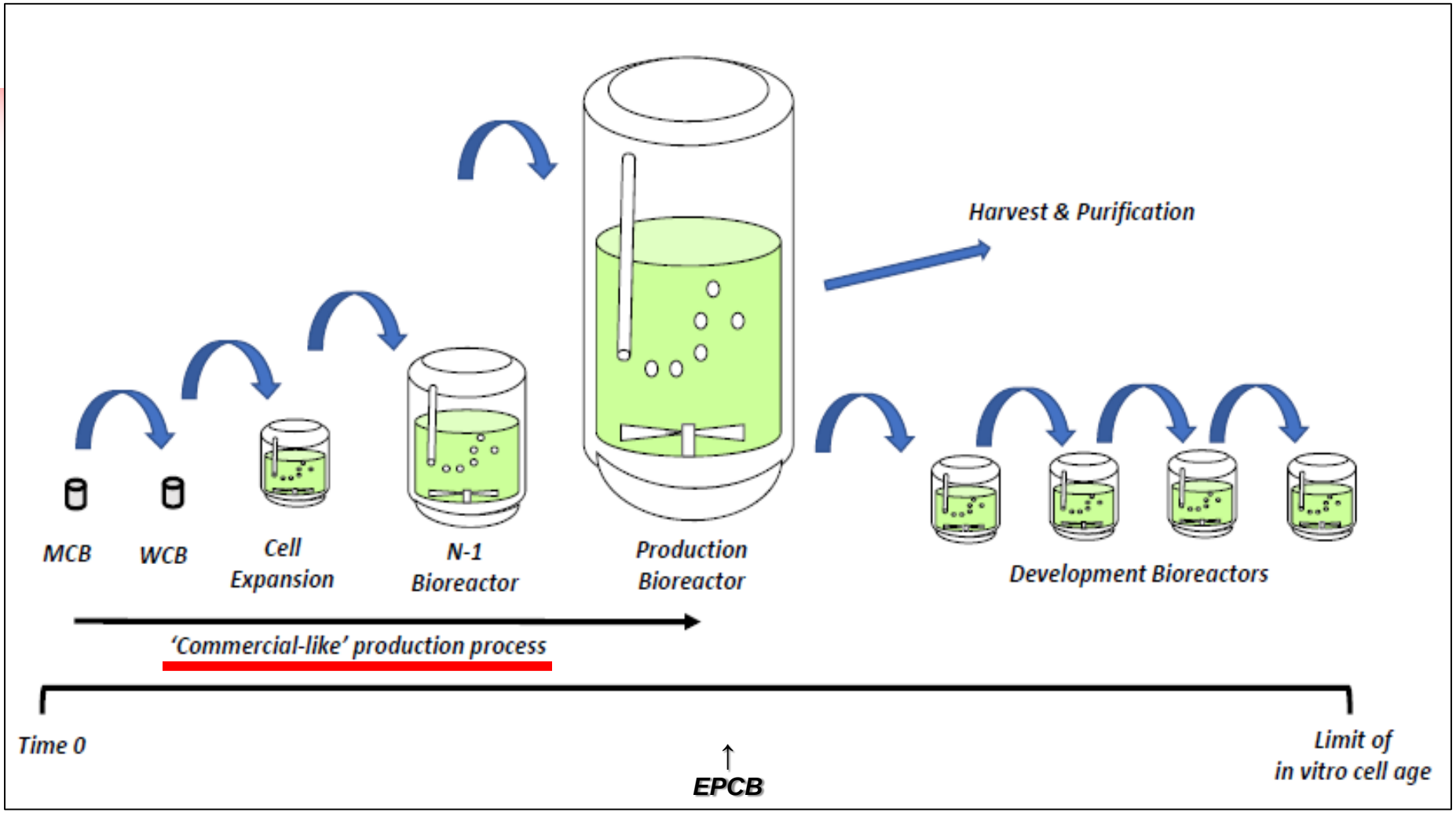


ICH Q5D/Q5A recommendations for genetic stability evaluation

- ***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)***

Inflectra MAb (Infliximab Biosimilar) EPAR Hospira 2013

Copy number loss – productivity impacted, but not product quality

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

Sp2/0 murine cells

Qarziba (dinutuximab beta) EPAR Apeiron Biologics AG 2017

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.

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

3 Major CMC Regulatory Compliance Issues of API Manufacturing

1) Genetic stability during the cell culture production process

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

Small-scale modeling studies are used extensively for biopharmaceuticals

Importance of small-scale manufacturing process studies for biopharmaceuticals

1) ***Number of Experiments Needed**: the more complex the process the greater the number of process parameters that need to be studied (even with DOE)*

2) ***Cost Savings**: expensive at full-scale to run a biopharmaceutical 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 biopharmaceutical 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) or are GMP inappropriate (e.g., bringing live viruses into the facility)*

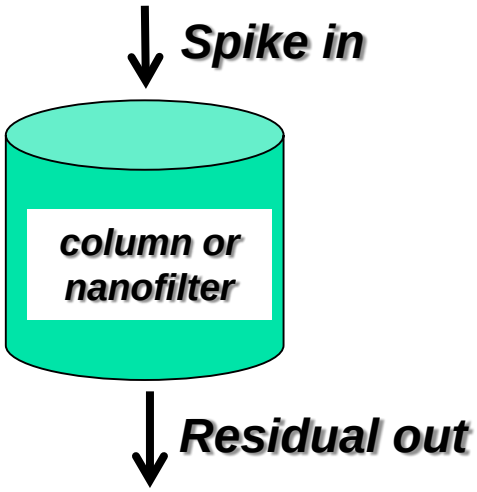
Scaled-down model studies are used across the biopharmaceutical manufacturing process!

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*



"All models are approximations"

British mathematician and statistician George E P Box

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

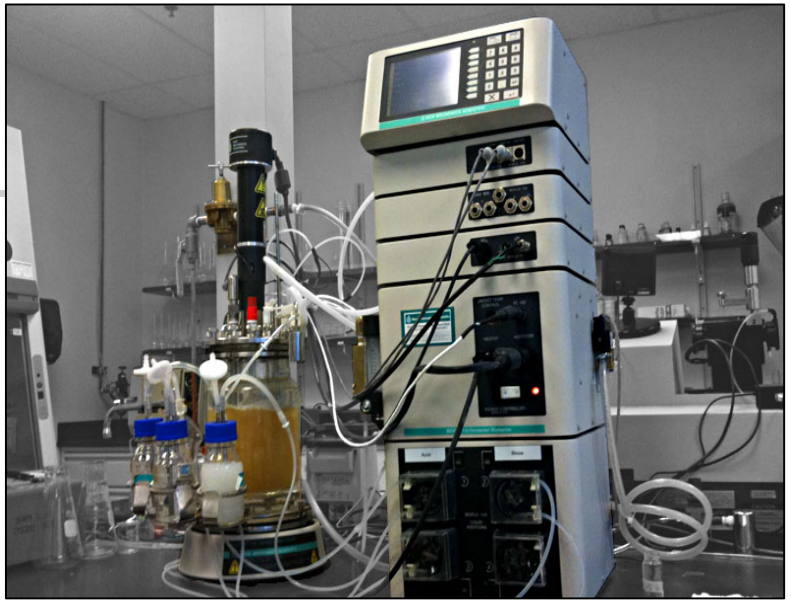
When used, small scale models should be described and their relevance for the commercial scale should be justified, in terms of objective, design, inputs and outputs. When validation studies are highly dependent on the small scale model studies (e.g. design space claimed), it may be necessary to demonstrate 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

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



Scaled-down models results need to be confirmed at full-scale!
(if at all possible)

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

***Expect* regulatory authority questioning of 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.

Chem Review

(No delay in standard 2+10 month FDA review)

3 Major CMC Regulatory Compliance Issues of API Manufacturing

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

3) Risk-based control of the API manufacturing process

Timing for the required process validation activities

Control of the biopharmaceutical 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

14 September 2017
EMA/CHMP/BWP/534898/2008 rev. 1

FDA
Stage 1 – Process Design

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
  - Media fill hold studies for aseptic processing

↓

Later Clinical Development Stage

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

FDA
Stage 2 – Process Qualification

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 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

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 process validation

EMA provides a guideline on process validation for biopharmaceutical 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 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'?

Video

Caution

FDA: '3 Run Rule' is Gone!

5. Do CGMPs require three successful process validation batches before a new active pharmaceutical ingredient (API) or a finished drug product is released for distribution?

No. ***Neither the CGMP regulations nor FDA policy specifies a minimum number of batches to validate a manufacturing process. The current industry guidance on APIs (see ICH Q7A for APIs) also does not specify a specific number of batches for process validation. FDA recognizes that validating a manufacturing process, or a change to a process, cannot be reduced to so simplistic a formula as the completion of three successful full scale batches.***

The manufacturer is expected to have a sound rationale for its choices in this regard. The agency encourages the use of science based approaches to process validation."

FDA Questions and Answers on Current Good Manufacturing Practices, Good Guidance Practices, Level 2 Guidance – Production and Process Controls; FDA website

ICH (FDA, EMA, JPMDA): '3 Run Rule' is Gone!

Generally, process validation includes the collection of data on an appropriate number of production batches (see ICH Q7, Section 12.5). The number of batches can depend on several factors including but not limited to: (1) the complexity of the process being validated; (2) the level of process variability; and (3) the amount of experimental data and/or process knowledge available on the specific process.

ICH Q11

So how many consecutive production batches will your company run for your biopharmaceutical process validation studies?

***Timing* for completion of process validation**

***MAJOR* difference between chemical drugs and biopharmaceuticals!**

Process validation can include the collection and evaluation of data, from the process design stage throughout production, that establish scientific evidence that a process is capable of consistently delivering a quality drug substance.

The drug substance manufacturing process should be validated before commercial distribution of resulting drug product. For biotechnological processes, or for aseptic processing and sterilisation process steps for drug substances, the data provided in support of process validation is included as part of the marketing application (3.2.S.2.5). For non-sterile chemical entity drug substance processes, results of process validation studies are not normally included in the dossier.

ICH Q11

Biopharmaceuticals – process validation must be completed with results reported in the submitted market application dossier!

Validation Studies for the Cell Growth and Harvesting Process.

A description and documentation of the validation studies which identify critical parameters to be used as in-process controls, to ensure the success of routine production should be submitted. Reference may be made to the flow diagram(s) as appropriate.

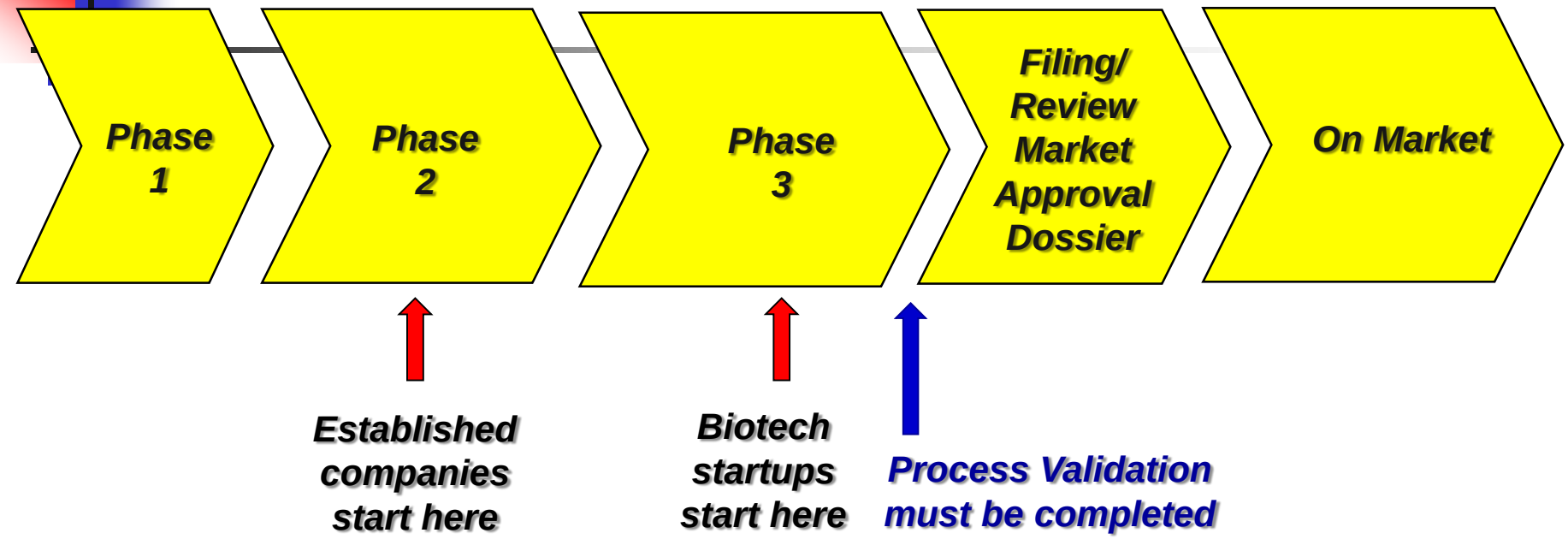
Validation Studies for the Purification Process.

A description and documentation of the validation of the purification process to demonstrate adequate removal of extraneous substances such as chemicals used for purification, column contaminants, endotoxin, antibiotics, residual host proteins, DNA, and viruses, where appropriate, should be provided. (See

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

Center for Biologics Evaluation and Research (CBER)
Center for Drug Evaluation and Research (CDER)
August 1996

Timing differences for starting process validation!



Earlier Process Validation Start

Pro – Once burnt, never again!

Con – Investment in $$ and resources for validation may either need to be repeated if the process changes or lost if the product fails clinical

Later Process Validation

Pro – Conserved $$ and resources for validation at later date

Con – Risk of surprises during process validation, and possible product approval delays

Biopharmaceutical process validation missteps!

3 Case Examples

- **Recombinant influenza proteins produced by insect cells**
  - The submitted process validation was incomplete, retrospective, and not supportive of a controlled manufacturing process – 4 year delay in FDA market approval
- **Monoclonal antibody produced by CHO cells**
  - The submitted process validation was insufficient and lacked validation protocols and reports – resulted in a 'major' amendment and added 3 months onto FDA review
- **Genetically engineered CAR T-cells**
  - Did not follow process validation guidance provided by the FDA during the pre-BLA meeting – repeated PV, no delay in market approval

FDA review of FluBlok

(insect cell/baculovirus produced influenza viral recombinant proteins)

BLA filed with FDA April 2008

Protein Sciences Corp received FDA Complete Response Letter August 2008

Please be advised that, based upon our review thus far, we do not concur with your assessment that the manufacturing process for the monovalent bulk drug substances has been validated at commercial scale. Identification of critical quality attributes and control of these attributes through the establishment of appropriate critical control parameters appears inadequate. Your process validation studies do not provide sufficient evidence of control of the critical quality parameters associated with the critical quality attributes. We recommend you review your process and consider additional parameters for testing.

The process validation presented by the manufacturer is poorly controlled and is in essence not a validation study but rather an attempt to demonstrate that product qualities are similar in different runs. Unfortunately not even this was demonstrated – immunogenicity of H3 in 2 of 3 drug product lots was less than immunogenicity of the 3rd lot. In describing this 'validation' study they state: ---------- ***retrospective not prospective*** ----------

FDA market approved Jan 2013

4 year delay

FDA review of Cosentyx (secukinumab)

CHO produced monoclonal antibody

Novartis

BLA submitted October 2013

FDA CMC Review

This BLA initially included little information regarding control of the manufacturing process. For example, non-critical attributes and key operating parameters were not included, it appeared that in-process limits could be changed without notification, development of the drug substance manufacturing process was not described and no data were provided, insufficient validation data were provided, validation protocols for (b) (4) were not included, and insufficient information regarding (b) (4) was provided, which could affect the acceptability of some aspects of the control strategy. In addition, critical quality attributes (CQAs) were not specifically identified.

CMC data that needed to be provided resulted in a 'major' amendment, extending the review timetable by 3 months

FDA market approved January 2015

FDA review of Kymriah (CAR T-Cells)

Genetically engineered cells

Novartis ***FDA Mid-Cycle Meeting*** ***May 2017***

Manufacturing process validation for Tisagenlecleucel - Based on the ongoing CMC review and results of the PLI at the Morris Plains NJ manufacturing facility, the following major CMC issues need to be resolved for approval of the BLA.

a. The product lots used for the process validation studies were manufactured before the validation protocol was formally approved by the Novartis quality unit and before the commercial process was established. This was not a prospectively designed validation study and is inconsistent with what FDA recommended during the pre-BLA meeting discussion.

b. Clinical batch records rather than commercial batch records were used for manufacture of lots used in the process validation study. FDA notes that there were multiple differences between the clinical batch record used at the time of the PV and the proposed commercial batch records.

c. Novartis did not run any batches with leukapheresis materials that contained high levels of monocytes as advised by the FDA during the pre-BLA discussion.

d. FDA questioned the acceptance criteria for critical process parameters (CPP) and key process parameters (KPP) used in the process performance qualification (PPQ) studies. Some of the CPP and KPP ranges are quite wide, and were based on data not submitted in the BLA. These ranges are sufficiently broad such that they would not help define a validated and controlled commercial manufacturing process. During the discussion with Novartis during the inspection, the FDA recommended that the acceptable ranges for CPPs and KPPs should be revised to reflect the accumulated manufacturing data and experience. FDA indicated that a simple 3 times the standard deviation may not be a suitable approach given the wide ranges of the available data.

e. Some unit operation holding times were not defined (e.g. (b) (4) , volume reduction, beads wash).

Novartis repeated process validation – no delay in market approval (August 2017)

Question

Can a biopharmaceutical process be considered 'validated' if 1 key manufacturing process step is out of control?

Genentech. **PERJETA™ (pertuzumab)**

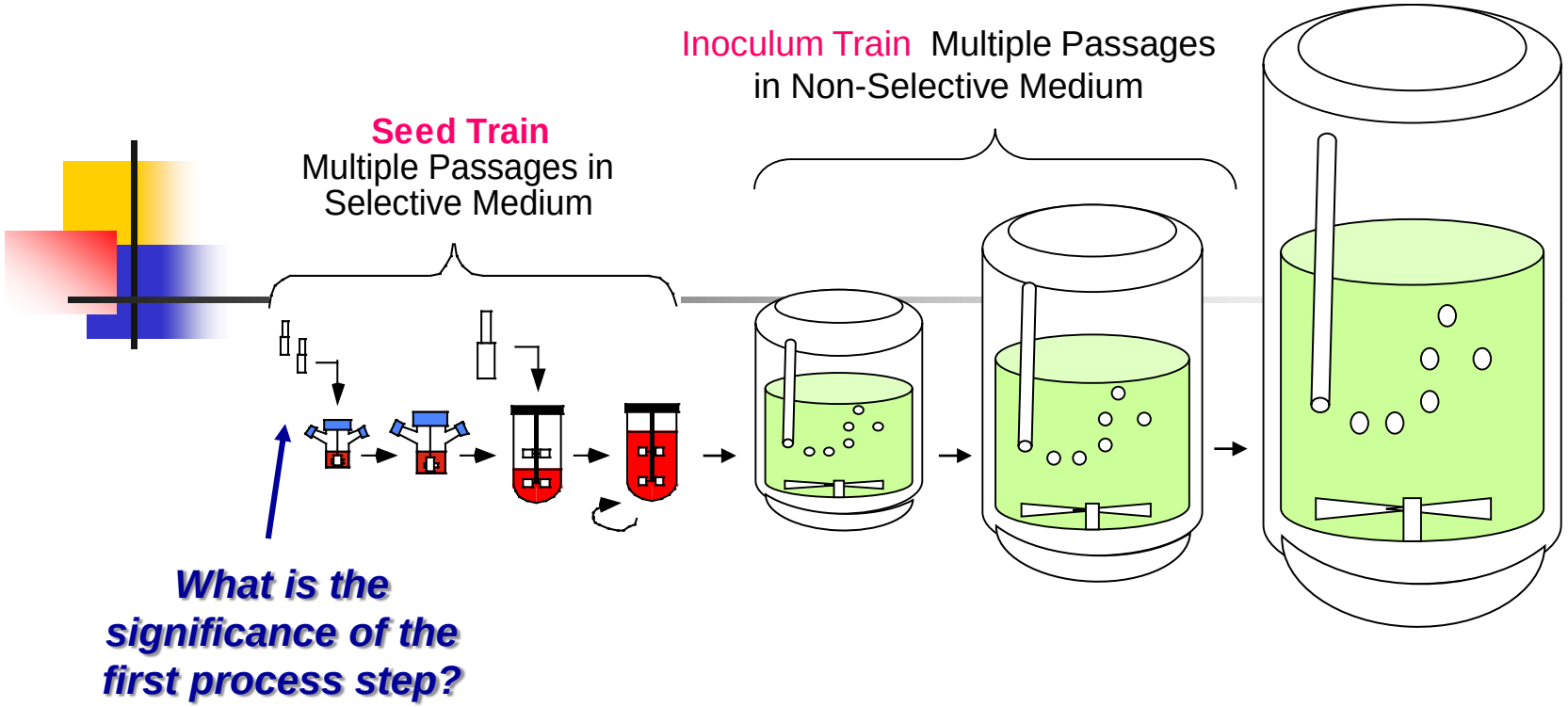
The Applicant has applied Quality by Design (QbD) principles to develop the process and product controls for the commercial manufacture of Perjeta.

Kathryn King (Traditional Elements Reviewer)
Laurie Graham (Quality by Design Reviewer)

Division of Monoclonal Antibodies

Concerns about the validation of the manufacturing process, discovered during the pre-approval inspection of the DS manufacture

A pre-approval inspection (PAI) for pertuzumab drug substance manufacture was performed at the Vacaville (VV), CA facility from March 20 to March 28, 2012 by BMT reviewer Bo Chi (lead), BMT trainee Qing Zhou, product reviewers Kathryn King and Laurie Graham and an inspector from the San Francisco District, Lance DeSouza. VV is responsible for the manufacture of pertuzumab drug substance and for DS QC testing. A form 483 was issued at the end of this inspection. Observations included: 1) The environment of (b)(4) facility where pertuzumab is manufactured is not maintained in a clean and sanitary condition; 2) There is a lack of assurance that water used in (b)(4) is suitable for its intended use; 3) Equipment cleaning validation studies are inadequate; 4) There is a lack of systematic oversight of the DCS (distributed control system) used to monitor and control process performance; 5) Quality oversight of documentation is inadequate; 6) There is inadequate control of raw materials. 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 (b)(4) 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



Summary Review for Regulatory Action

The initial and continued major concern in regard to this issue is whether Genentech has a validated process and can consistently manufacture pertuzumab with product quality characteristics comparable to that used in their clinical trials. Given the ongoing failures with the current working cell bank, Genentech has not yet demonstrated a consistent process that would ensure continued supply of commercial material.

CHEMISTRY REVIEW(S)

The Division of Monoclonal Antibodies (DMA), Office of Biotechnology Products, OPS, CDER, does not currently recommend approval of STN 125409 for Pertuzumab manufactured by Genentech. The data submitted in this application are inadequate to support the conclusion that the manufacture of Pertuzumab is well controlled and consistently leads to a product that is pure and potent.

Based on the understanding that the applicant has refused to make this product more widely available to patients prior to licensure while the manufacturing issues are being addressed, the clinical review office has indicated their intent to approve this product within a time frame consistent with the PDUFA deadline and to resolve outstanding manufacturing issues post-licensure. To the knowledge of the CMC review team, the initial licensure of a biological product under a BLA without concurrent approval of the manufacturing facility and the manufacturing process is unprecedented. This approach was agreed upon by the CDER Director. Therefore, DMA participated in the drafting of PMRs as the only mechanism available to mitigate risks to product quality from a process which lacks adequate validation.

Last minute FDA higher up intervention – Telecon June 07, 2012, one day before PDUFA clock and market approval

Genentech

Josephine, Ing, Sr. Scientist, Regulatory Affairs
Mark "Kip" Benyunes, Senior Group Medical Director, Product Development Oncology Clinical Science
Dietmar Berger, Vice President, Clinical Development, Hematology/Oncology
Ian Clark, Chief Executive Officer, Genentech and Head of North American Commercial Operations
Michael Doherty, Senior Vice President, Global Head Product Development Regulatory
Liz Homans, Vice President, HER2 Franchise, Global Product Strategy
Sandra Horning, Senior Vice President, Global Head Clinical Development Hematology/Oncology
Josephine Ing, Regulatory Program Director, Product Development Regulatory
Karen Jones, Global Head Oncology, Product Development Regulatory
Lynne Krummen, Senior Director, Pharma Technical Regulatory
Theresa Martinez, Lifecycle Leader, Global Product Strategy
Teresa Perney, Director, Product Development Regulatory
Michelle Rohrer, Vice President, US Regulatory Affairs, Product Development Regulatory
Mary Sliwkowski, Vice President, Regulatory Chemistry Manufacturing and Controls and Information Systems
Pascal Soriot, Chief Operating Officer, Roche Pharmaceuticals Division
Patrick Yang, Executive Vice President, Head Global Technical Operations

FDA

Janet Woodcock, Director, CDER
Richard Pazdur, Director, OHOP *(Office of Hematology/ Oncology Products)*
Robert Justice, Director, DOP1 *(Division of Oncology Products)*
Amna Ibrahim, Deputy Director, DOP1
Patricia Cortazar, Clinical Team Leader
Gideon Blumenthal, Clinical Reviewer
Nancy Scher, Clinical Reviewer (Safety)
Kathryn Fedenko, Deputy Director Safety
Denise Esposito, Deputy Director, ORP
Maryll Toufanian, Associate Chief Counsel for Drugs, OCC
David Joy, Regulatory Counsel, ORP/DRPI
Elizabeth Giaquinto, Project Manager, OEP
Mary Beth Clarke, Acting Director, OEP *(Office of Pharmaceutical Science)*
Helen Winkle, Director, OPS
Steven Kozlowski, Director, OPB *(Office of Biotechnology Products)*
Patrick Swann, Deputy Division Director, DMA
Kathryn King, Biologist, DMA
Patricia Hughes, Team Leader, Microbiology Product Quality, OC/OMPQ/BMAB
Bo Chi, Ph.D., CMC Microbiology Reviewer, OC/OMPQ/DGMPA/BMAB
Steven Lynn, Director (Acting), OMPQ
Ilisa Bernstein, Deputy Director, OC
Tara Gooen, LCDR, Acting Chief, OC/OMPQ/DGMPA
Mahesh Ramanadham, LT., Acting Team Leader, OC/OMPQ/DGMPA
Tamy Kim, Associate Director of Regulatory Affairs (Acting), IO/OHOP
Alice Kacuba, Chief Project Management Staff, DOP1
Amy Tilley, Regulatory Project Manager, DOP1

Extraordinary load on the process validation group!

Commitment for 3 concurrent PV studies mentioned in market approval letter next day

4. Conduct a process validation study to support manufacture of pertuzumab from the Master Cell Bank. Submit the Final Report as a PAS.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

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

5. Conduct a process validation study to support manufacture of pertuzumab from a new Working Cell Bank. Submit the Final Report as a PAS.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	04/2013
Study Completion:	09/2014
Final Report Submission:	10/2014

6. Conduct process validation studies to support manufacture of pertuzumab from Working Cell Banks by a modified process. Submit the Final Report as a PAS.

Conjugating the Biopharmaceutical API

Antibody-Drug Conjugates (ADCs)

(>60 ADCs in clinical study)

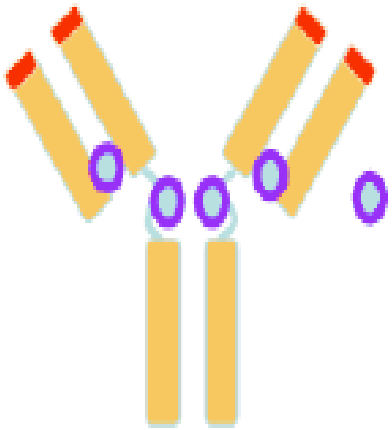
Antibody-Drug Conjugates (ADCs)

ADCETRIS (brentuximab vedotin)

Antibody — **Linker** — **Chemical Drug**

cAC10 anti-CD30 antibody	Attachment group	Protease-cleavable linker	MMAE cytotoxic drug

(~ 4 MMAE molecules/MAb molecule)



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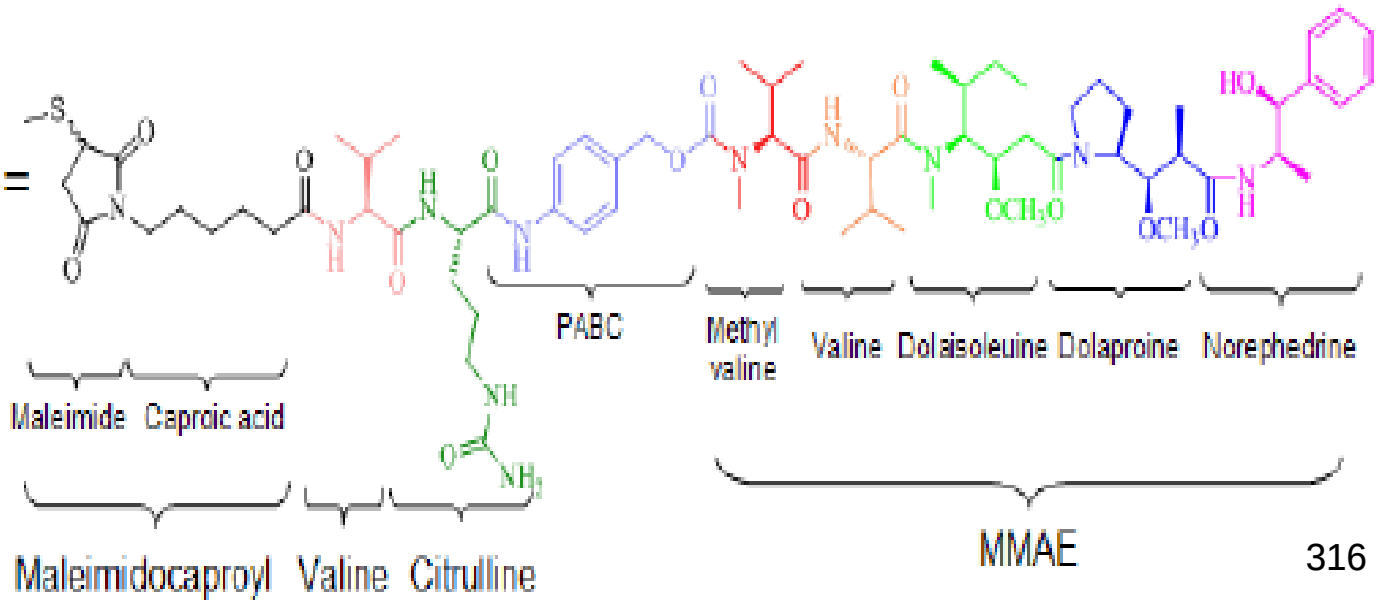


ADCs require addressing BIOLOGIC mAb CMC concerns

ADCs require addressing CHEMICAL DRUG CMC concerns

- ***Manufacture of highly cytotoxic chemical drugs (toxins)***
  - ***Worker safety***
  - ***Chiral purity***
  - ***Residual organic solvents (ICH Q3C)***
  - ***Residual elemental impurities (ICH Q3D)***
  - ***Mutagenic impurities (ICH M7)***
- ***Both the toxin and the chemical linker need to be manufactured and tested under appropriate and adequate GMP-like control***
- ***Typically, the toxin and chemical linker are chemically combined before attachment to MAb***

***TOXINS* currently incorporated into commercial ADCs**

Calicheamicin

MYLOTARG
BESPONSA

- Most potent naturally occurring hydrophobic antibiotic cytotoxin
  - Approximately 1000-fold more active than doxorubicin against xenograft tumors
- Binds to the minor groove in DNA, resulting in double stranded DNA breakage and cell death

Maytansine

KADCYLA

- Potent anti-mitotic macrolide with clinical activity in broad range of tumors
  - Synthetic maytansine analogs, DM1 and DM4
- Inhibits mitosis by interfering with microtubule assembly

Auristatin

ADCETRIS

- Highly potent fully synthetic analog of natural product, dolastatin-10
  - MMAE (membrane permeable)
  - MMAF (membrane impermeable)
- Inhibits mitosis by interfering with microtubule assembly

Duocarmycin

- DNA alkylating agent, picomolar activity
- Binds to DNA minor groove, resulting in DNA alkylation and cell death

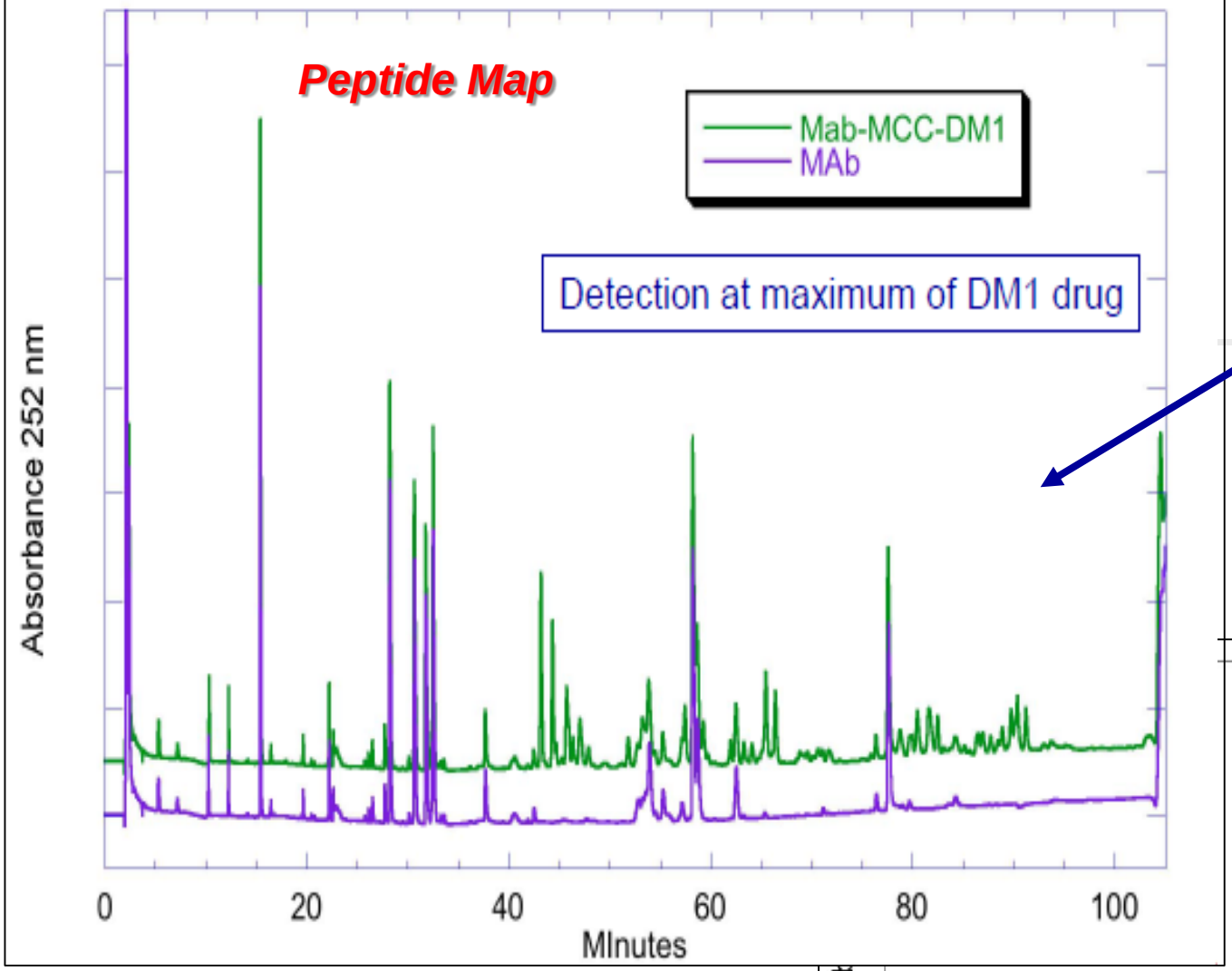
Pyrrolobenzodiazepine (PBD)

- Class of naturally occurring anti-tumor antibiotic found in Streptomyces, sub-nano/picomolar activity
- Binds to DNA minor groove, PBD dimers cross-link opposing DNA strands producing highly lethal lesions

ADCs require addressing ADC CMC concerns

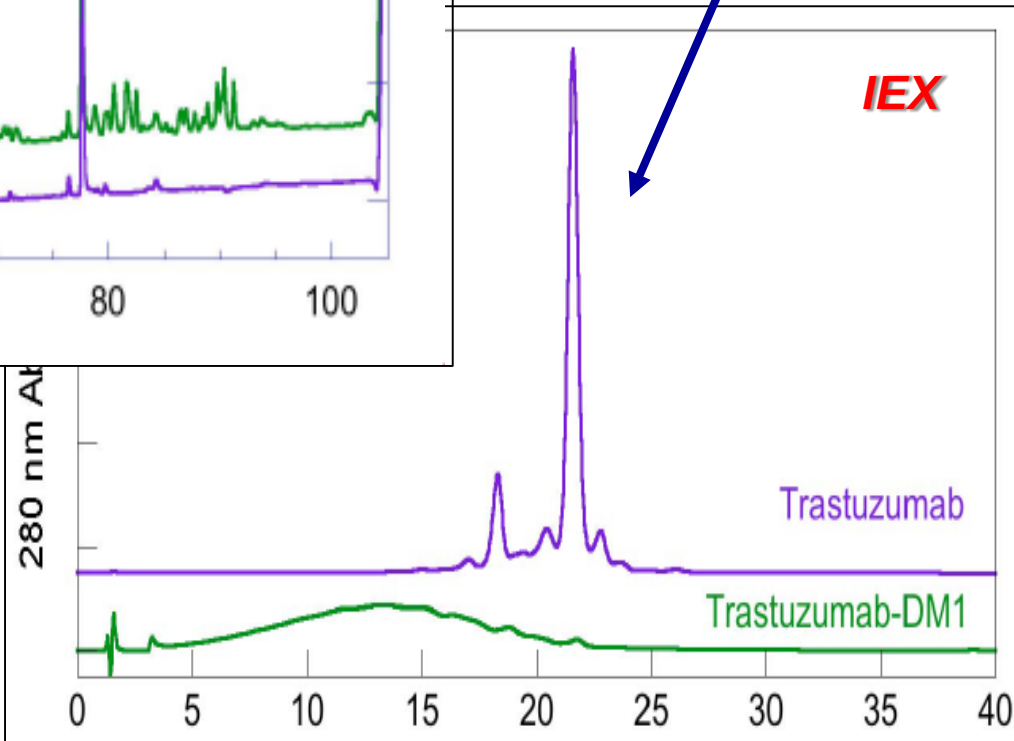
- ***Assuring ADC lot-to-lot manufacturing consistency***
  - ***Adequate and appropriate control of the chemical reaction conjugation process ensuring consistency of the number of toxin molecules per MAb molecule***
  - ***Residual free toxin (and unconjugated MAb)***
- ***Assuring ADC lot-to-lot stability***
  - ***Linker instability (e.g., hydrolysis)***
  - ***Toxin instability (e.g., oxidation)***
  - ***MAb instability (e.g., aggregation)***

Challenge: conjugation of drugs to mAbs can cause a loss of analytical characterization power



Increased peptide complexity after conjugation

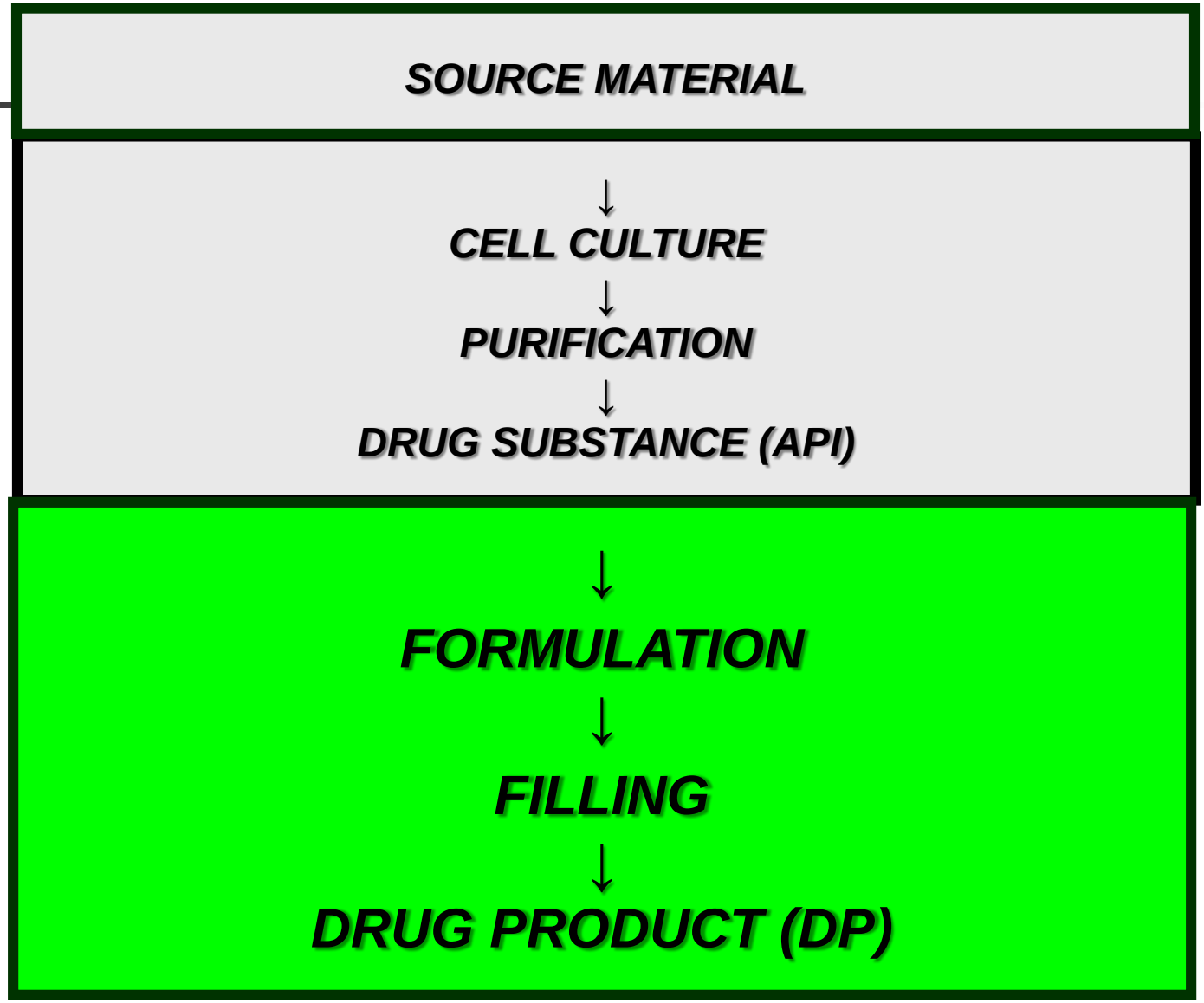
Peak resolution loss after conjugation



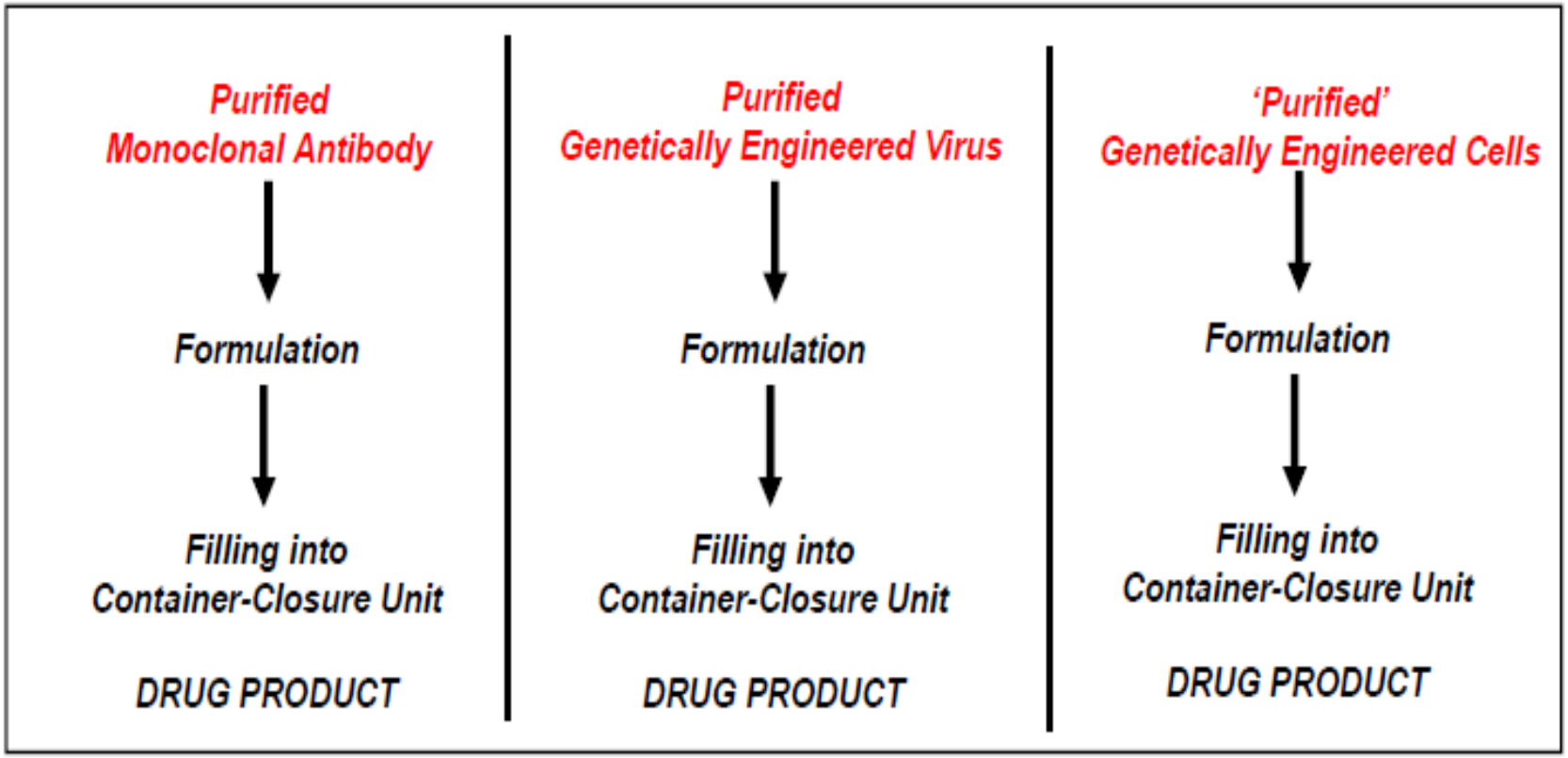
F. Jacobson, Genentech
WCBP 2007

Basic Manufacturing Process Flow Diagram

Application of CMC Risk-Managed Control Strategy



Drug product manufacturing for biopharmaceuticals

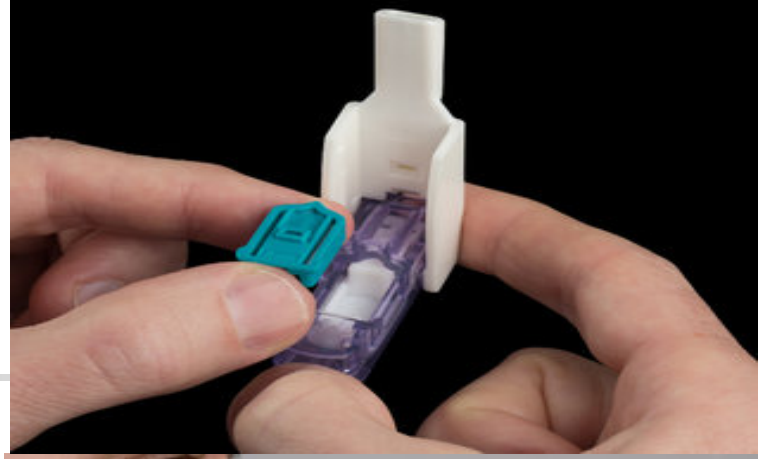


Biopharmaceuticals are formulated with excipients

but every added excipient needs to be justified

- ***Minimization of molecular variant formation***
- ***Stability of bioactivity/functionality***
- ***Solubility of product***
- ***Bulking agent (if lyophilized)***
- ***Cryoprotectant (if frozen)***
- ***Antimicrobial preservative***
- ***….***

High approval threshold for Novel Excipients
(an excipient being used for the first time in a drug product, or by a new route of administration; regulatory region specific)



Novel Excipient in Afrezza

Human Insulin formulated with FDKP

Central to the functionality of Afrezza is the excipient fumaryl diketopiperazine (FDKP)

FDKP imparted the critical 0.5-5.8 micron particle size for inhalation

Anything bigger than that impacts in the back of the throat
Anything smaller than that is exhaled

FDKP treated as a novel excipient
2 yr tox study

Illustration of the required formulation development studies required for market approval

22 June 2017
EMA/CHMP/559383/2017

Formulation development

In the developmental stage, formulation development studies were performed to confirm the effects of pH, buffer, excipient, and protein concentration on the stability of Imraldi finished product. The formulation development studies and the results were presented. From the results of the developmental studies above, the following conclusions were drawn for optimised Imraldi formulation. Finished product formulation robustness study was done to assess the formulation robustness of Imraldi finished product with variation of protein concentration, pH, L-histidine concentration and sorbitol concentration. Additionally, optimal formulation composition range was identified through this study. Results of the developmental robustness study showed that the Imraldi finished product formulation is robust within range of protein concentration, pH, and L-histidine concentration. The overall results of the formulation robustness study indicate that the formulation may be sufficiently robust at the proposed storage conditions, and that the protein concentration and pH are important factors to ensure acceptable quality of the finished product throughout the shelf-life.

Study done on same formulation as Humira

Commercial formulations are being successfully changed!

Case Example of Market Approved Biopharmaceutical

(Rituxan/MabThera monoclonal antibody)

Original IV formulation: 10 mg/mL rituximab in sodium chloride, sodium citrate and polysorbate 80

↓

New SC formulation: 120 mg/mL rituximab in L-histidine/ histidine hydrochloride, trehalose, polysorbate 80, L-methionine, and recombinant human hyaluronidase

Case Examples of Market Approved Biosimilars

Sandoz's biosimilar of Neupogen (G-CSF): Changed to glutamate buffer (pH 4.4) in place of acetate buffer (pH 4.0) used by Amgen

Sandoz's biosimilar of Enbrel (anti-TNF): Changed to citrate buffer in place of phosphate buffer used by Amgen

But not all commercial formulation changes are successful!

Dash of EDTA!

- ***Leukine (rh GM-CSF) was originally approved by the FDA in 1991 for Immunex; Immunex also developed a liquid formulation which the FDA approved in 1995 [I was VP Q at the time]***
  - ***Leukine was then passed from company to company when Amgen purchased Immunex, but didn't want Leukine***
- ***In 2006, Bayer, the new owner of Leukine, received FDA approval to add a 'touch' of EDTA to the liquid formulation***
  - ***"EDTA, a chelating agent, approved by the FDA as a preservative in vitamins and baby food, traps metal impurities and thereby extends the shelf life of organic products — making it a logical adjunct to a protein based therapeutic such as Leukine."***

- ***But only 2 years later, in January 2008, Bayer voluntarily withdrew liquid Leukine after post-marketing safety reports indicated an upward trend in adverse events, in particular, that of syncope (fainting)***

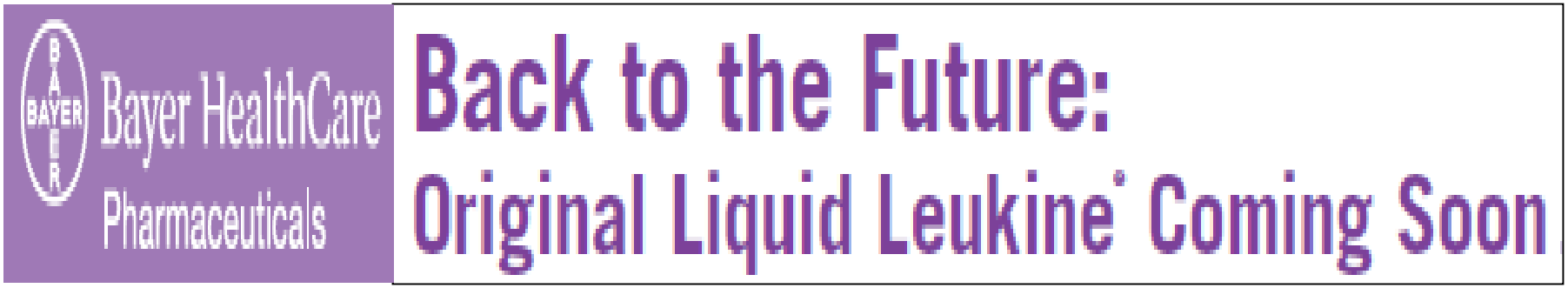
- ***Investigation revealed:***
  - ***"The addition of EDTA appears to increase the absorption rate of GM-CSF, the active ingredient in Leukine, and may result in a temporary increase in plasma concentration of GM-CSF shortly after administration"***

Sometimes it can take months or years in commercial use, before a change in an adverse event profile can be confirmed

(This is the reason why regulatory authorities consider biologic formulation changes to be a 'high risk')

- ***Took Bayer 5 months to take EDTA back out of the liquid formulation – May 2008***
  - ***"FDA has approved Bayer's reintroduction of a formulation of liquid Leukine (sargramostim) that does not contain EDTA"***

A+ to their Marketing Department:



Container-Closure Unit

Biopharmaceuticals are typically, but not exclusively, delivered parenterally (i.e., by injection)

Parenteral

- *Glass vial with rubber stopper*
- *Pre-filled syringe*
- *Auto-delivery needle device*
- *Pre-filled plastic administration bag (cells)*

Inhalation

- *Aerosol nebulizer (Pulmozyme recombinant human DNase)*
- *Dry powder inhaler (Afrezza recombinant human insulin)*

Topical

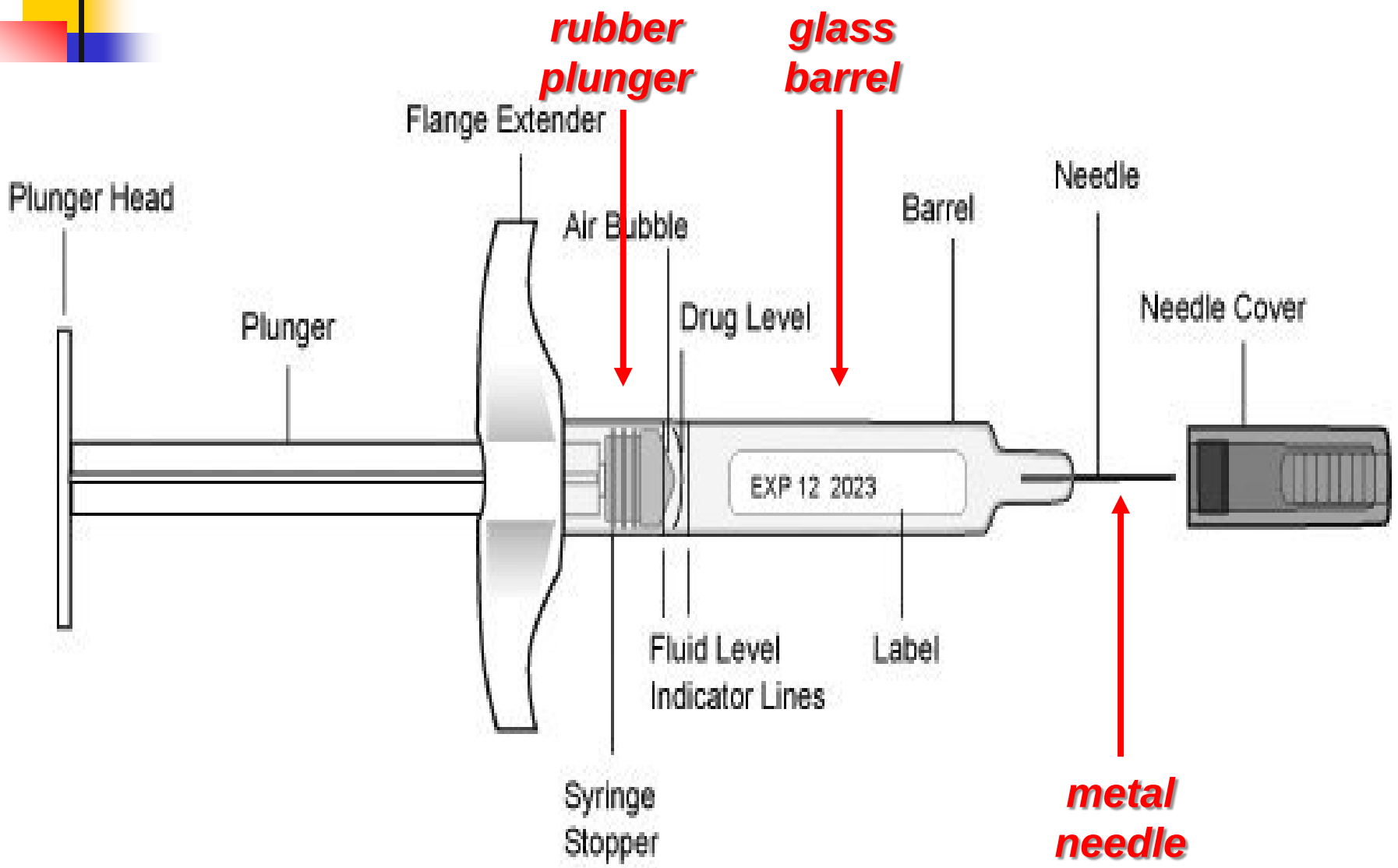
- *Transdermal gel (Regranex recombinant human PD growth factor)*
- *Eye drop (Oxervate recombinant human nerve growth factor)*

Rectal

Vaginal

Oral

Biopharmaceuticals are not inert to product-contact surfaces from the container-closures

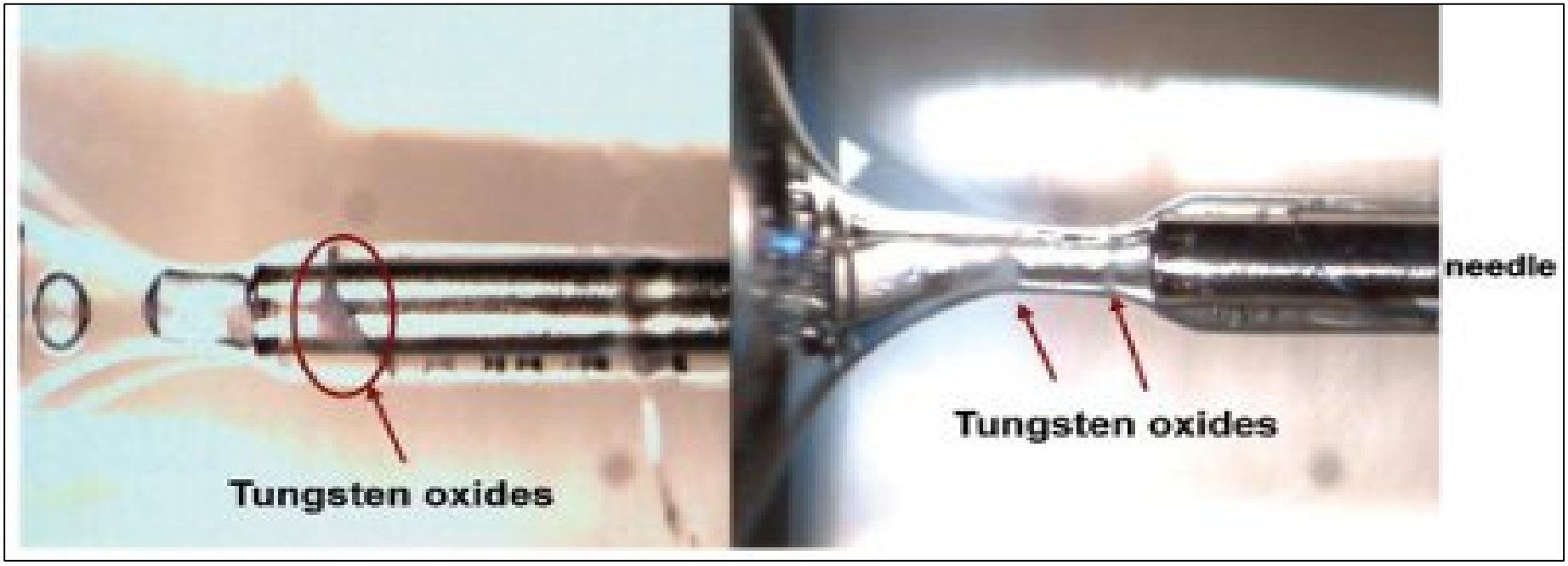


Discovery of tungsten oxides in pre-filled syringes

Tungsten ion accelerates protein aggregation

During glass syringe manufacture, while the glass barrel is being formed at high temperature (at 1200°C), a tungsten pin is used to shape and maintain the hole where the stainless steel needle will be glued in

During pin removal, residual tungsten ion can remain



Improved syringe washing processes at the vendors

Incoming batch check for residual tungsten (ICP/MS)

Shocking discovery of glass vial delamination

Micro-Flow Imaging (MFI)

(counting and photographing each type of particle present)

Glass lamellae

Potentially present in every glass vial of Epogen manufactured since 1982!

AMGEN

Recall September 2, 2010 Epogen (epoetin alfa)

RECALLING FIRM/MANUFACTURER	RECALLING FIRM/MANUFACTURER
Recalling Firm: Amgen Inc., Thousand Oaks, CA	Recalling Firm: Centocor Ortho Biotech, Inc., Horsham, PA
VOLUME OF PRODUCT IN COMMERCE	VOLUME OF PRODUCT IN COMMERCE
78,074,450 vials	16,759,926 vials

Delamination does not occur in pre-filled glass syringes

(vials are formed at ~1400°C, while syringes are formed at ~1200°C)

Vial manufacturing process can minimize the problem of delamination – molded process vs tube process (molding uses lower temps than tube)

Avoiding unbuffered solutions and avoiding high pH can minimize glass delamination

Container-closures (other than vial-stopper) are DEVICES

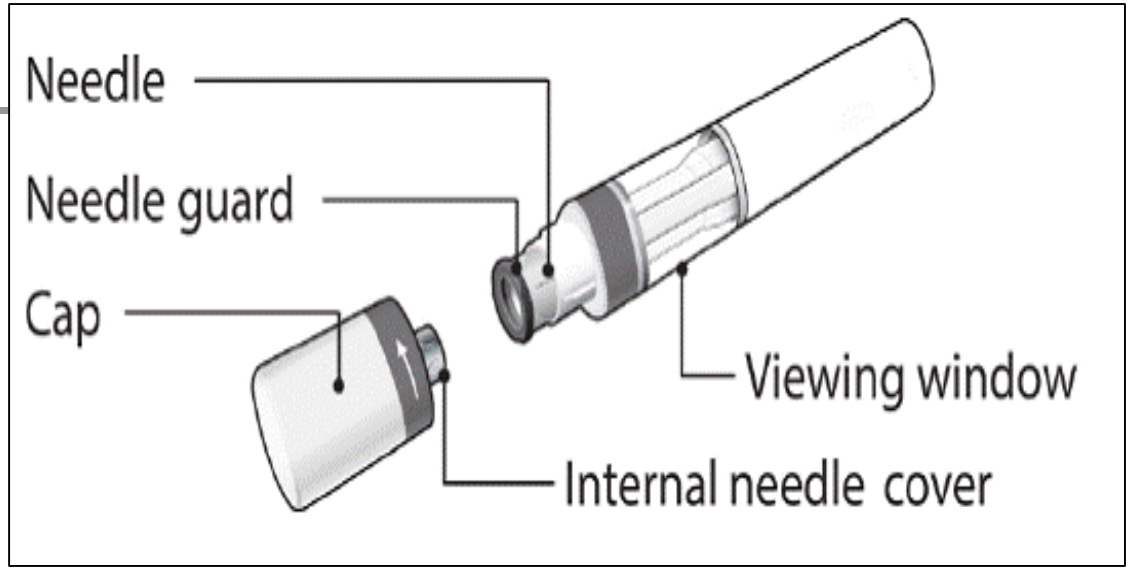
device (in addition to biologic) regulations must be met

Case Example

Erelzi (etanercept-szzs) Injection

Confirmation of device operation is necessary!

(both upon release and at end of shelf life)



Develop methods for confirming the injection depth (e.g. needle length exposed for injection), audible feedback (e.g. occurrence of second click) and visual feedback (e.g. plunger fills the window and stops moving) for release testing. Define release specifications that meet design output specifications for injection depth, audible feedback, and visual feedback for lot release testing prior to launch of Erelzi. Submit the study report and release specifications in the annual report.

Case example where device design ('usability study') delayed market approval of a biopharmaceutical

REGENERON

October 25, 2018 at 7:00 AM EDT

Regulatory Update on EYLEA Pre-filled Syringe

Regeneron also announced today that the U.S. Food and Drug Administration (FDA) issued a complete response letter (CRL) regarding the Chemistry, Manufacturing and Controls (CMC) Prior-Approval Supplement (PAS) for the EYLEA pre-filled syringe. The CRL requested additional information regarding manufacturing and supply processes and the completion of a usability study evaluating a single injection of the EYLEA pre-filled syringe in approximately 30 patients. Regeneron expects to compile all the requested information and resubmit the PAS in early 2019 and continues to expect a 2019 launch of the EYLEA pre-filled syringe.

Human engineering studies are most important!

In an emergency, do you know which end to push into the skin?

Life saving for hyperglycemia

Life saving for anaphylactic shock

If someone can do something dumb with your device, it will happen!

Unique CMC challenges of Cell-Based biopharmaceuticals

FDA approved 2017

CAR – chimeric antigen receptor

2 ***CAR T-cell genetically engineered cells***

Whenever a new biopharmaceutical type makes it commercially …

… some will start saying 'the sky is falling'!

Cell and Gene Therapies: Industry Faces Potential Capacity Shortages

GEN
Genetic Engineering & Biotechnology News
November 15, 2017

Gene Therapy Hits a Peculiar Roadblock: A Virus Shortage

The New York Times
NOV. 27, 2017

We may soon have our first $1 million drug. Who will pay for it? And how?

PBS NEWS HOUR
Oct 15, 2017

… and there always will be 'rogue ventures'!

- **Self injection of gene therapies**

Why I injected myself with an untested gene therapy - BBC News
www.bbc.com/news/world-us-canada-41990981
Nov 21, 2017 - The moment Tristan Roberts became the first human to **inject** an untested, experimental **gene therapy** into his stomach fat, he was sitting on a …

A biotech CEO explains why he injected himself with a DIY herpes …
https://www.technologyreview.com/.../a-biotech-ceo-explains-why-he-injected-himsel...
Feb 5, 2018 - Traywick's stunt is the latest example of **self-injection** by biohackers who, despite … Biohackers Disregard FDA Warning on DIY **Gene Therapy**.

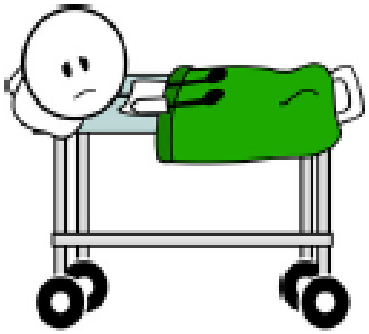
- **Stem cell 'false promises'**

American CryoStem Corporation **WARNING LETTER** January 3, 2018

… your firm receives and processes adipose tissue, a structural tissue, for autologous use … your firm isolates cellular components from the adipose tissue, thereby processing the adipose tissue into Stromal Vascular Fraction (SVF). The SVF is then expanded through cell culture to produce your product ATCELL™. American CryoStem then ships the autologous product back to physicians to treat patients for a variety of diseases or conditions by various routes of administration, including intravenously, intrathecally (i.e., injection or infusion into the central nervous system) and by aerosol inhalation

… records reveal that ATCELL™ is intended to treat a variety of diseases and conditions, including, but not limited to, anoxic brain injury, Parkinson's disease, amyotrophic lateral sclerosis (ALS), stroke, and multiple sclerosis.

… always a learning curve!



vein to vein, 2-3 weeks

Black Box In

1 batch = 1 patient

Black Box Out

Cell culturing manufacturing process

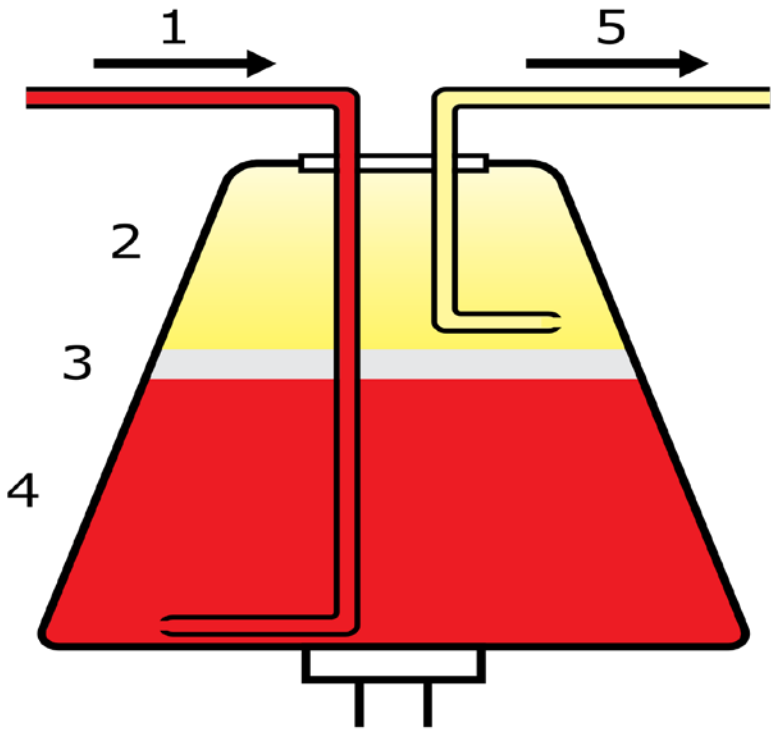
Substantial manipulation of cells → Cellular Therapy
(if genetic engineering involved → Gene Therapy)

Industry knows how to handle cells!

Learning Curve: inconsistent source material

consistency of incoming patient cells impacts CQAs

– *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

Ways to minimizing inconsistency from cell collection

- ***Obtained not by GMP 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."*
>
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> 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***

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