

Regulatory Consideration for Biotechnology Products: Clonality of the Production Cell Bank

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Overview

- Regulatory expectations for assurance of clonality of the production cell bank used in the manufacture of Biotech products
- Commonly used cloning procedures and the recommended supporting data to provide assurance of clonality of the production cell bank
- Mitigating risk to the product lifecycle from lack of assurance of clonality of cell bank
- Case Studies: Resolution of issues related to lack of assurance of cell bank clonality

***Regulatory Expectations for
Assurance of Clonality of
Production Cell Bank***

Regulatory Consideration: Production Cell Bank for manufacture of Biotech Products

A well-characterized production cell bank is a fundamental building block in the manufacture of a therapeutic Biotech product.

Assurance of production cell bank clonality ensures consistency of product quality and process performance throughout the lifecycle of a product

- A clonal cell bank is better at tolerating seemingly innocuous changes in cell culture conditions e.g., cell culture raw material, change in cell culture conditions, unforeseen changes that may favor preferential proliferation of a subpopulation

Risks to the Lifecycle of a Product due to Lack of Assurance of Clonality

A change in product quality that may lead to uncertainty and need for additional clinical /non-clinical studies

or

Impact process performance negatively

or

Inability to predict the risk associated with “minor” process changes

Regulatory Expectation of a Clonal Cell Line

“For recombinant products, the cell substrate is the transfected cell containing the desired sequences, which has been cloned from a single cell progenitor.” -ICH Q5D

“Monoclonal antibodies are immunoglobulins (Ig) with a defined specificity derived from a monoclonal cell line.”

“The cell substrate to be used for the production of the monoclonal antibodies should be a stable and continuous monoclonal cell line...” - GUIDELINE ON DEVELOPMENT, PRODUCTION, CHARACTERISATION AND SPECIFICATIONS FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS (EMA/CHMP)

Regulatory Consideration for Production Cell Bank

Following information should be included in the regulatory submission to support the clonality of the production cell bank:

- History of the host cell line used in the manufacture of the MCB
- Vector and Transfection/Transduction method
- Cloning procedure used in the generation of the MCB
- Information on the stage at which the cells were adapted to suspension and serum free/ chemically defined conditions as well any subsequent cloning steps
- How clones were expanded, evaluated and selected for cell banking
- If an animal derived reagents used during cloning procedures, a risk assessment should performed on potential exposure of the cells to adventitious agents.

Regulatory Consideration: Adaptation of Cell Line

Forced adaptation to culture conditions can potentially lead to drift in genotype and phenotype of a cell therefore,

- Cell should be cloned after adaptation to growth in suspension or serum free/ chemically defined culture
- If cells are adapted post-cloning, additional cloning should be done to assure clonality of the final cell line

Considerations for Commonly Used Cell Cloning Procedures

Considerations for Commonly Used Cloning Procedures

Limited Dilution Cloning

- Two rounds of limiting dilution cloning (LDC) at “sufficient dilution level” are considered acceptable.

Cell Sorting (FACS) and Imaging Technologies:

- FACS isolate single cells using size, fluorochrome or surface marker. The sorted cell are seeded and expanded to select a clone for MCB.
- FACS alone may be sufficient or can be combined with one round of LDC
- Data to support clonality should include but not limited to the description of how the cells were sorted e.g., instrument settings, imaging of wells to verify cell/well.

Considerations for Commonly Used Cloning Procedures

Soft Agar/Methylcellulose (e.g., ClonePix)

- Cells are plated at low density in semisolid media to promote individual colony Growth.
- Provides rapid screening of high producer clones.
- Use of two rounds of ClonePix or one round of ClonePix with LDC is recommended to provide an acceptable probability of clonality.
- Data to support the clonality should include parameters used for colony selection, technique used to recover the individual colonies from the agar

Consideration for commonly used Cloning Procedures

Clone Select Imager

- Single cells are plated and imaged from day 0 until the cell grows into a colony
- Supporting data should include picture of a single cell and the entire well

Isogenic Cell Lines

- An isogenic cell line is engineered so that the construct of interest will integrate into the same location in every cell
- For licensure, it is recommended that isogenic cells bank should be created in the same way as non-isogenic lines and have an equivalent assurance of clonality

***Mitigating Risk to Product
Lifecycle from Lack of
Assurance of Clonality***

Mitigating Risk of Lack of Assurance of cell Bank Clonality on Product Lifecycle

Due to lack of understanding of the causes of variations of cells in culture, there is no reliable method to model growth characteristics of cells in culture. The logical alternative controls include:

- The starting material: production cell banks
- Cell culture process controls and control of raw materials
- Additional product characterization

Mitigating Risk of Lack of Assurance of Cell Bank clonality on Product Lifecycle

When the clonality of the MCB is in doubt, the following additional testing should be considered to provide increased assurance of clonality:

- fluorescence in situ hybridization to evaluate the individual integration sites or a sub-clone analysis where a vial of the master cell bank is plated as single cells, expanded, and characterized and,
- sub-clone analysis where a vial of the master cell bank is plated as single cells, expanded, and characterized using phenotypic analysis (e.g. cell doubling time, specific productivity etc.), product quality testing

Mitigating Risk of Lack of Assurance of Cell Bank Clonality on Product Lifecycle

Deficiency in providing a high assurance of cell bank clonality may result in augmentation of control strategies to mitigate risk during commercial production. An augmented control strategy will include, but might not be limited to

- Limits on in vitro cell age, EOP characterization
- Additional in-process monitoring of upstream process
- Enhanced testing for each lot of drug substance e.g., monitoring sequence variant, glycosylation
- Additional controls for the WCB qualification protocol
- Additional risk assessment of any changes made in critical raw materials, including media and media components.

Mitigating Risk of Lack of Assurance of Cell Bank Clonality on Product Lifecycle

Acceptability of lack of assurance of clonality of a production cell bank is dependent on adequacy of the final control strategy (CS):

- High probability of Clonality → Acceptable
(no additional CS required)
- Low probability + High Assurance (additional testing)
→ Acceptable (no additional CS required)
- Low probability + Little Assurance + Augmented CS
→ Acceptable

Case Studies: Common Clonality Issues

Case Study #1

A product under development, used single round of ClonePix cloning procedure. Information on the generation of the master cell bank using ClonePix was very limited and was not sufficient to provide assurance of clonality of the production cell bank.

A non-hold comment was sent to update IND with detailed information on the ClonePix FL clone selection method including, but not limited to,

- The size of the colonies selected, the probability of clonality at that size,
- The technique used to recover the individual colonies from the agar and,
- Controls in place to prevent adventitious agent contamination of the selected clone. Adventitious agent information should include information on the steps taken to prevent cross-contamination of products during the selection process and information on the quality of the labeled Protein A/G reagent used for cell selection.

Case Study #1-continued..

Company's Response

Detailed description of the methodology was provided as requested in the information request

Additional data to support clonality of the cell banks, cells from MCB and WCB were analyzed using fluorescence in-situ hybridization (FISH) and multicolor FISH (mFISH).

The FISH technique was used to detect the presence of specific DNA sequences, e.g. GOI for the antibody on chromosomes and to characterize the karyotype features (chromosome morphology and number) of a given cell.

mFISH used to visualize the reconstitution of chromosomes when rearrangement occur and to identify specific chromosome which include GOI for the antibody.

The data to support the clonality of the MCB are deemed acceptable

Case Study #2

Cloning Procedure: Following transfection, cells were sub-cloned by one round of limited dilution at high cell density under selective pressure followed by second step cloning using FACS to seed 1 cell/well for cloning MCB

No supporting data was included in the submission to assess probability of clonality

Information Request:

- Provide information on the FACS procedure and reagents used.
- Provide available data to support the expected efficiency of the cloning method e.g., microscopic evaluation following seeding or studies performed to qualify the instrument and protocol.

Case Study #2 contd...

Company's Response

- FACS Instrument settings were described to provide assurance of cell sorting procedure and microscopic evaluation was performed 12 days after FACS to verify seeding density of 1 cell/well.
- Additional data to support the qualification of FACS instrument was also provided from another cell line where a cell Imager was used following FACS. According to the sponsor same documented procedures were used for both cell lines.

The data to support the clonality of the MCB was deemed adequate

Case Study #3

Cloning Procedure: Single round of Limited dilution at “0.5 cell/well” with media adaptation post cloning

- Two sublines (A/B) in MCB/WCB identified in late product development stage
 - Different number of chromosomes
 - Different expression cassette loci possibly due to chromosome rearrangements
- Ratio of the sublines varies batch to batch without apparent assignable cause
- Extensive product characterization of product batches derived from cultures (predominantly A; predominantly B; and A/B mixture) showed consistency of CQA

Case Study #3 contd....

Proposed strategy to manage the Clonality and consistency of the product quality during product lifecycle:

- Perform additional characterization on all DS during manufacture
 - Glycosylation pattern
 - Enhanced analysis of peptide map ID test
- Use statistical process control for continued process verification to identify potential drifts
- Additional in process testing for the qualification of the WCB Comparability protocol for release of batches manufactured using the new WCB
- Post marketing commitment to identify the difference in the two subpopulation with respect to growth characteristics and product quality

Case Study #4

Master cell bank was cloned using single round of LDC using 5 cells/well.

FDA Comment: Cloning procedure does not provide sufficient assurance of clonality of the MCB. The lack of assurance of clonality of the therefore, provide additional data to support the clonality of the master cell bank.

Company's Response: The company has no direct evidence clonality. To support clonality of the MCB the company provided following:

- Characterization data (genetic stability and phenotype) of the MCB, WCB, and end of production cell bank
- Data showing consistent process performance and product quality from the number of DS batches manufactured using different working cell banks

Case Study #4 contd....

the data provided did not address the impact of different manufacturing conditions and are, therefore, not robust enough to support lifecycle management of the product.

Submit supplemental data to support the assurance of clonality of the MCB. Such data could include sub-clone analysis where a vial of the master cell bank is plated as single cells, expanded, and characterized

- using phenotypic analysis (e.g. cell doubling time, specific productivity etc.), product quality testing, and genotypic analysis (e.g. fluorescence in situ hybridization or southern hybridization) to evaluate the individual integration sites.
- The lack of assurance of clonality will have significant impact on the control strategy and the type of studies that will be required to make even minor changes to the manufacturing process.

Summary

- Clonality of the production cell bank should be established early in the product development
- Sufficient information should be provided to assess the capability of the method used to produce a clonal cell line
- Methods used for acquiring supporting data to assure clonality of the cell line should be sufficiently sensitive and suitable for the purpose
- Adaptation of the cells should be performed prior to cloning or a cloning step should be performed after adaptation to culture conditions

Summary

- If the cloning procedure used in the manufacture of production cell bank doesn't provide sufficient assurance of clonality additional testing on the subclones derive from production cell banks should be performed to provide assurance of clonality e.g.,
 - genotype (FISH for expression cassette, host cell karyotype) and phenotype analysis (cell doubling time, specific productivity etc.) and product quality analyses.
- In the absence of the supporting data on assurance of clonality, an enhanced control strategy for the monitoring upstream process, DS batch release and additional studies may be required for even a minor process change

Thank You !