Regulatory Considerations in Establishing Clonality for Cell lines Expressing Therapeutic Proteins

Audrey Jia, M.D., Ph.D. M.S.
Former FDA Biologic Product CMC Reviewer in Office of Biotechnology Products

5th Annual Cell Line Development & Engineering Asia, may 16 -19, 2016, Shanghai, China
Outline

- Regulatory expectations for clonality of cell banks
- Cell subcloning methods and their use in supporting the clonality
- Examples of FACS single cell sorting to support the clonality
- Cases of clonality issues
Guidance

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

- FDA Points to Consider in the Characterization of Cell lines Used to Produce Biologics (1993): The MCB is defined as a collection of cells of uniform composition derived from a single tissue or cell.

- EMA/CHMP Guidance on Development, Production, Characterization and Specifications for Monoclonal Antibodies and related Products (2008): The cell substrate to be used for the production of the monoclonal antibodies should be a stable and continuous monoclonal cell line that has been developed by means of recombinant DNA and/or other suitable technologies.
Regulatory Comments for Clonality

• Please update the IND with details, including data to support the clonality assessment of your MCB.

• The IND does not include sufficient information on the master cell bank (MCB) manufacturing process to provide assurance of clonality.

• ...Update the IND with more detailed information on the FACS-based clone selection methods, including controls that are in place to assure that a colony derived from a single cell is selected and any other assessment that may be performed to address the assurance of clonality.
The Ideal Cell line

• Stable
• High producing
• Express the product with the desired quality: especially important for the biosimilar development
• Scalable
• *Clonal*
Truth of the Mammalian Cell Culture

• There are no absolute homogeneous cells in the bioreactor (from 1 ml of cryovial to 2,000 even 20,000 liters of bioreactor, with the immortal cell lines that tend to mutate).

• Cells used in biopharmaceutical manufacturing processes are inherently prone to genotypic and phenotypic drift.

• Selection pressure could also impact the stability of protein expression in cells.

• Recombinant proteins produced by living cells are highly complex and inherently heterogeneous molecules.

• One can not demonstrate clonality, rather, can use data to show that your cell line development methods can assure that MCB has a high probability of being clonally-derived.
## Results of Cell Phenotypes After 3 Rounds of LCD

<table>
<thead>
<tr>
<th></th>
<th>MCD (X10^5 cells/mL)</th>
<th>Growth Rate (X10^5 cells/mL/D)</th>
<th>Doubling Time (Hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st round of LDC (mean)</td>
<td>19.45 ± 1.44</td>
<td>4.85 ± 0.34</td>
<td>25.90 ± 1.77</td>
</tr>
<tr>
<td>1st round of LDC (range)</td>
<td>9.65 - 28.45</td>
<td>2.42 - 6.96</td>
<td>22.02 - 32.85</td>
</tr>
<tr>
<td>2nd round of LDC (mean)</td>
<td>24.30 ± 1.39</td>
<td>6.30 ± 0.29</td>
<td>23.25 ± 1.09</td>
</tr>
<tr>
<td>2nd round of LDC (range)</td>
<td>16.30 - 30.70</td>
<td>4.45 – 8.01</td>
<td>19.25 – 30.74</td>
</tr>
<tr>
<td>3rd round of LDC (mean)</td>
<td>23.78 ± 0.88</td>
<td>5.68 ± 0.43</td>
<td>23.17 ± 1.20</td>
</tr>
<tr>
<td>3rd round of LDC (range)</td>
<td>18.15 – 28.15</td>
<td>3.27 – 8.13</td>
<td>16.70 -28.80</td>
</tr>
</tbody>
</table>

Inherent Feature of Cell Culture

- Phenotypic change or drift are noted in growth rate, doubling time and maximum cell density.
- May impact the stability, production and product quality of the secreted recombinant proteins.
- The variation in growth patterns decreased after subcloning.
- Cells may have selective regulatory mechanism that prevent extreme variation within a population.
The Importance of Ensuring Clonality of MCB

- To minimize the variations in product quality caused by the mixed clones: products from clonal derived cell line are less variable than those from non-clonal derived cells.
- To minimize the genetic and phenotypic diversity within a cell line population
- To ensure the consistency of the process and product: it is a part of the overall control strategy.
- To minimize the risk of the manufacturing change: if change causes shift of product quality, at least cell bank can be removed as a potential cause for investigation
- Especially to biosimilar product development: to minimize the burden of proofing “highly similar” with nonclonal cell banks.
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/nonclinical studies
• Impact process performance negatively
• Inability to predict the risk associated with “minor” process changes
Current Methods Used for Cell line Generation and Subcloning

• Limiting dilution: classic, < 0.5 cell/well, 2 rounds
• ClonePix: high expressing colonies
• FACS single cell sorter: one cell per well
• Cell imaging instruments that allow high-throughput scanning of microwell plates and generate images of the freshly plated cells
• Next Generation Sequencing (NGS)
ClonePix

• Detailed methods need to be submitted to the Agency
• The size of the colonies selected, and the probability of clonality at that size
• The technique used to recover the individual colonies from the agar
• Controls in place to prevent adventitious agent contamination of the selected clone.
• Should include information on the steps taken to prevent cross-contamination of the products during the selection process and information on the quality of the labeled protein A/G reagent used for cell selection.
FACS Single Cell Sorting

- FACS procedure and reagents used
- Set up the machine to optimize the operation of dropping one cell per well: exclude doublets, aggregates, cell morphology, cross-contamination, and safety concerns from animal derived reagent, etc.
- Need system development studies
- Expected efficiency of the cloning methods: demonstrate of single cell per well by using cell imager
- Sorted cells into multiple 96 well plates
- Using plate imaging system to visualize and count wells with 0 cells/well, one cell/well, and > 1 cell/well. Calculate the possibility of a cell/well using statistical analysis
Combination of the Methods to Support the Clonality

• Two rounds of limiting dilution at < 0.5 cell/well: classical method, acceptable.
• One round of limiting dilution at < 0.5 cell/well with cell imaging technology
• FACS single cell sorter alone with cell imaging
• FACS single cell sorter with one round of limiting dilution at a suitable density
• ClonePix with one additional round of limiting dilution at a suitable density
• Two rounds of ClonePix at defined conditions
Requirement for Imaging

• Protocol of cell imaging
• Capability of the Imaging system:
  1. Requirement for imaging: spin off or not
  2. Sensitivity at day 0
  3. Edge effect
  4. Imaging entire wells or not
• Supporting data should include picture of a single cell and the entire well.
Time Course of Cell Imaging from Day 0 to Day 12

Day 0

Day 1
Day 2
Day 3
Day 4

Thomas Sterovsky, Data generated on Solentim Cell Metric, from Informa Cell Line Development Conference, April 2016
Examples of Good Imaging to Support the Clonality

Day 0

Day 1

Courtesy of Solentim
Documentation of Clonality Reporting
Clonality Reporting

1. Meets FDA requirements of whole well image and where single cell feature is located
2. Allows highlighting of other features which can then be described away e.g.
   • Debris – in the plate pre-cells being added
   • Or does not grow or change over the following days
3. Carries raw untampered data
4. Select days to be included in report e.g. Day 0, 1, 2, 3, 7
5. Carries full audit trail with time and date stamp

From Solentim Inc
Generating Product with Consistent Quality

• Clonal MCB
• Robust manufacturing in process controls
• Meaningful release specification to control the DS/DP
• Lifecycle management
Examples of Product Quality Impact due to Clonality issues

• Changing in raw materials lead to a significant change in the product quality in potency. Root cause indicated that the cell bank is not clonal. Need additional nonclinical and clinical study to support the change.

• Changing the growth medium caused the less representative population growing faster and generating product with different glycosylation profile. Not comparable, sub-cloning again.

• Transfection and plating at a high cell density, followed by FACS single cell sorting: need to provide FACS sorting methods, controls, and cell imaging and/or microscopic evaluation results to support the clonality.
Data to Support the Possibility of Clonality in Submission

• Host cell line used in the establishment of MCB
• The vector information, transfection procedure, selection and cloning procedures
• Any adaptation and subcloning procedure performed: If cells are adapted to grow in serum free medium, additional subcloning steps are required.
• Detailed information on the clone selection for PCB and MCB
• Qualification data for your subcloning methods, including the instrument set-up, and operational checks
• Statistical analysis to demonstrate the possibility of clonality
• Always: animal sourced materials and their risk control
• For cells obtained by site-specific method: the cell bank should be created in the same way as those derived from random integration and have an equivalent assurance of clonality.
If Lack of Assurance of Cell Bank Clonality

• FISH to check the integration sites
• Sub-clone analysis: a vial of MCB is plated as single cells and perform phenotypic analysis (e.g. cell doubling time, specific productivity etc.), product quality tests
• Additional control strategies to mitigate risk during commercial production including, but not limited to:
  1. limits on in vitro cell age, EOP characterization
  2. additional in-process monitoring of upstream process
  2. enhanced testing for each lot of DS: aa sequence, glycosylation etc.
  3. additional controls of WCB qualification protocol
  4. risk assessment of any changes in additional raw materials, including medium and medium components.
• Acceptability of lack of assurance of clonality depends on adequacy of the final control strategy

Copied and modified from Dr. Rawat presentation in Vienna 2016. Informa Life Sciences Annual Cell Line Development and Engineering Conference
Changing Cell Banks During Development

• A comparability study is required to bridge the product depending on when the change is implemented.
• Whether the original MCB is clonal or not.
• Changing to a different MCB vs. changing WCB using the same MCB
• At Phase 1 vs Phase 3: risk is higher at later development phase
• Follow ICH Q5E: analytical, nonclinical and clinical
Conclusion

• Clonality is a regulatory requirement that need to be fulfilled to support your drug development.
• Regulatory bodies recommended clonality be established early in the product development.
• Regulatory requirements for control strategy of manufacturing changes to products derived from clonal cell banks and non clonal cell banks are different.
• Regardless the cell cloning methods used, sufficient information should be provided to assess the capability of the cloning method used to produce a clonal cell line.
• The methods need to be qualified for their capabilities to ensure high possibility of clonality.
• If lack of assurance of clonality, additional genotype and phenotype analysis and product quality tests are needed.
• An enhanced control strategy for monitoring upstream process, DS batch release and additional studies may be required for even a minor process change.
Contact Info:
Email: jia_audrey@yahoo.com
Phone: (001)-301-801-3271
And linkedin
Acknowledgement

Solentim Inc. for the cell imaging pictures and documentation reports.