Establishing Clonal Cell Lines – A Regulatory Perspective

Black Cell, Blue Cell, *Old Cell*, *New Cell*?

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Disclaimer

The views and opinions expressed in this presentation should not be used in place of regulations, published FDA guidances, or discussions with the Agency.

Objective

Highlight some of the current FDA concerns regarding:

- Single cell cloning procedures
- Assurance of monoclonality

Provide examples of insufficient information or probability of monoclonality

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)

Limiting Dilution

- Historically have expected two rounds of limiting dilution at a sufficient dilution level
  - Advances in technology (e.g., imaging) might allow for one round of cloning performed at an appropriate dilution when combined with this technology.
  - Observing one final colony is not the same as imaging at day 1.

- Poisson Distribution is typically used to calculate probability of clonality.
  - This calculation is useful; however, it is important to consider that this calculation does not take cell behavior into account (e.g., cells are “sticky”).
  - There is no “magic” number.

Limiting Dilution – Examples
from BLA/IND submissions

- 90% probability of clonality with no supporting data/information is not sufficient.
- One round of cloning at one cell per well is not sufficient.
- Three cells per well does not equal one round of limiting dilution cloning.
- “Plating efficiency” (back calculating the percent of wells in which colonies grew) cannot provide assurance of clonality.

Soft Agar/Methylcellulose

- e.g., Clonpix
- Potential to pick more than one colony
- One round of cloning is likely not sufficient without extremely good supporting data
  - Need to account for cell behavior (cell-cell interactions)
  - Example of 58-87% probability of clonality from one sponsor
  - Have been provided examples of non-clonal lines
- Animal-derived reagents (e.g., antibodies, BSA in antibody storage buffer)
  - Expectations regarding adventitious agents are the same as for any other animal-derived reagent used in manufacturing of the cell banks or products.

FACS-based

- How is the system set to sort single cells?
  - Differentiate between one cell and two small cells (exclusion of doublets)
- Supporting system development studies
  - We expect data to demonstrate the ability to select single cells
    - Data provided have been from mixing studies. Studies should be done with cells that have been cultured together to be more representative of true cell cloning conditions.
    - Viability/stickiness at beginning and end of sorting
    - Does the level of aggregation affect the ability to exclude doublets?
    - Does variability in cell morphology affect ability to identify single cells? What is variability in morphology of cells (and difference between cells used for any development studies and the production cell line)?
- Animal-derived reagents (see previous slide)
- Contamination from equipment used with multiple cell lines

Capillary Aided
("spotting" and single cell transfer systems)

Need evidence of single cell status

- "Visual confirmation by 2 scientists of the presence of a single cell in a 1 μl droplet" is not sufficient.
  - Can images be captured? Acceptability of images from microscopic examination would be dependent on imager information.
- Public data (Onadipe, et. al, Lonza Biologics poster)
  - Small number of positive wells analyzed (156)
  - Appears to be cell mixing study (not co-culture)
  - A number of wells assessed as 0 cells showed growth of colonies.
  - These data would not provide sufficient assurance of clonality.

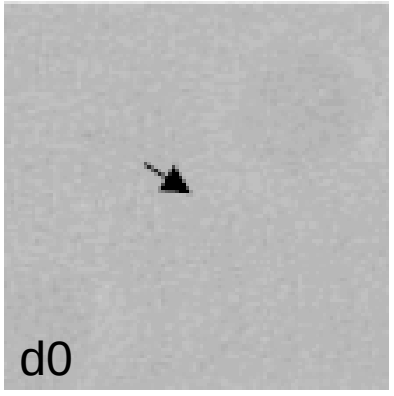
Other Techniques

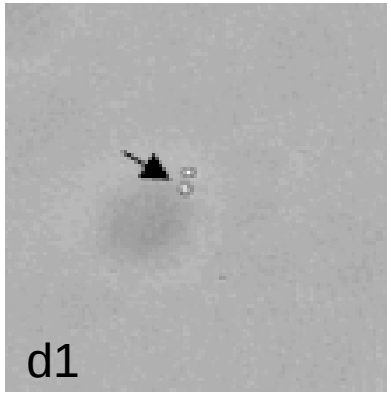
- Cloning rings/disks
- Gel microdrop

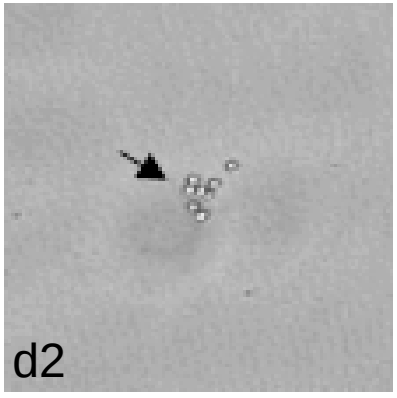
Recommend discussion with the Agency

Imaging

- What is the capability of the imaging system?
  - Will all cells be detected? Cells at edges of wells?
    - Do plates need to be spun down/what are requirements for cell position in wells?
- Single colony image is not sufficient (single cell image needed)
- Image(s) of entire well needed







Is there 1 cell or 2 cells at d0?
Can the system detect an image that I can't see? How well?

Support for clonality

- Genetic stability ≠ clonality
- Sequencing of gene of interest ≠ clonality
- Integration sites provide better information regarding clonality, if integration is not site-specific.
- Methods used for supporting studies need to be qualified to demonstrate sufficient sensitivity.

If a cell bank is subcloned for the purpose of supporting clonality:

  - A sufficient number of subclones need to be analyzed.
  - Subcloning and testing should be performed on cells as close to the original cloning as possible, to ensure that one line does not out-compete another slower growing line(s).

Adaptation of Cells

- Cells should be cloned **after** adaptation to growth in serum free conditions and/or suspension culture.
- If cells are adapted post-cloning, additional cloning should be done to assure clonality of the final cell line.
- Forced adaptation can lead to changes that are not compatible with a clonal cell line. (e.g., glycosylation changes; Costa, A.R., et al., New Biotechnol. 2012)

If clonality has not been assured

- Additional controls on manufacturing and other aspects of the product quality control strategy will be required.
- For example:
  - Greater than “typical” amount of characterization to assure that comparable product is generated from all subsequent working cell banks manufactured
  - Control over potential variation in product quality that could occur when there are “small” changes in the manufacturing process that would not typically be studied in detail and submitted to the agency
    - Requires submission of identification of “high” risk changes and risk mitigation and control strategies

If clonality has not been assured

- Additional controls on manufacturing and other aspects of the product quality control strategy will be required.
  - (from an **IND**): Control strategies that would ensure that DS (and any antibody intermediate) and DP manufactured in the future would be sufficiently consistent
    - Typical release specifications utilize a limited array of criteria that are appropriate for a product derived from a clonal cell bank, but it is not clear that the same specifications would be able to identify and control for the variability that may arise if the ratio of the various clone populations changed, such as could occur when using a new lot of raw material or following relatively minor changes in the growth or fermentation process.
    - Control strategies other than lot release testing should also be identified.

Conclusions

- Many methods (***old and new***) can be used to generate clonal cell lines; the methods should be qualified for their capabilities and be described in detail in the submission.
- Adaptation should be performed prior to cloning.
- Sufficient information/data should be provided to verify the capability of the method used to establish a clonal line.
- Methods for acquiring supporting data should be sufficiently sensitive and suitable to the purpose.

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