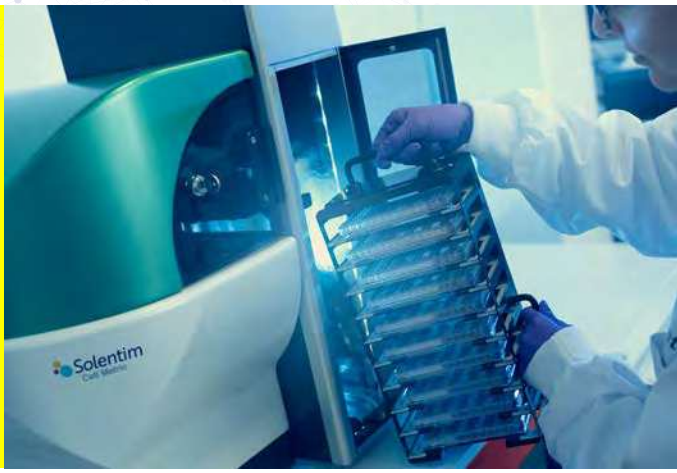
# Reducing the timeline by 30% to produce a monoclonal cell line expressing a clinical candidate bi-specific antibody




## Introduction

SystImmune Inc., based near Seattle (WA), has its own antibody discovery platform and their objective is to generate a single cell clone with maximal expression level of their candidate bispecific immune-oncology antibody, which is targeted to the treatment of solid tumour cancer.

## Cell Line Development at SystImmune

SystImmune decided to use the CHOZN® (Sigma) cell line as their expression platform. The CHOZN® ZFN-Modified GS-/-CHO cell line has the endogenous Glutamine synthetase (GS) knocked out, rendering the cells auxtrophic for the essential amino acid L-glutamine and hence making the selection process very simple.

Along with the CHOZN Platform, SystImmune also chose to adopt the minipool selection procedure for selection of higher producing clones. Whilst this may have taken longer to set up in the beginning, the belief was that it would generate an overall higher-producing cell line.

In addition to the CHOZN platform and minipool selection method above, SystImmune have implemented seeding by limiting dilution, along with the Solentim Cell Metric® system, into their process to obtain clonally-derived cell lines along with supporting documentary evidence for their IND submissions.

With all of these tools in place they are able to successfully screen and select for a lead single cell clone, producing at least 1 gram per liter, for Research Cell Banking (RCB) from a large starting population of minipool transfectants.

## Overview of the Cell Line Development Process

SystImmune have developed a comprehensive Cell Line Development (CLD) process with multiple stages as outlined in Figure 1.

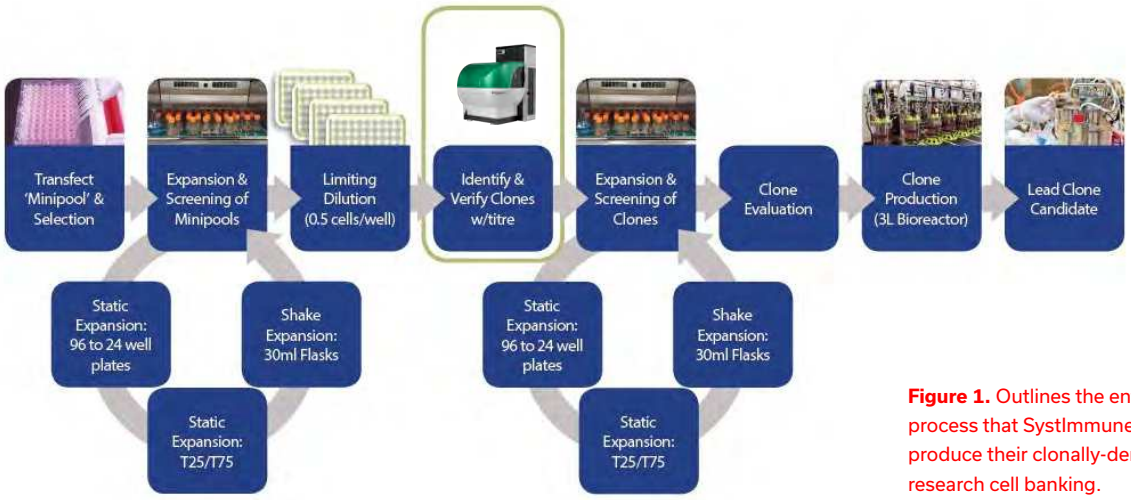



**Figure 1.** Outlines the entire cell line development process that SystImmune have adopted to produce their clonally-derived cell lines for research cell banking.

## Minipool Transfection & Selection Procedure:

CHOZN cells are transfected with a vector containing the gene of interest (GOI), then seeded at 5,000 - 10,000 cells per well. Recovery rate is very high due to the CHOZN system; for example, if 2-5 plates are seeded then the recovery rate is approximately 80%. At 14 days there are large numbers of wells containing multiple colonies. As it is difficult to expand all of the minipools that grow, a 7 day titre assay is carried out to eliminate any minipools that didn't express.

## Expansion and Screening of Minipools:

The top 50 minipools are expanded (via static and shaker vessels) and screened for titre. Static expansion involves expanding minipools from 96 well plates to 24 well plates and then from T25 to T75 flasks. Shaking expansion involves expanding and adapting the minipools to 30ml fed batch shaker flasks for the production run. SystImmune carry out this 30mL fed-batch shake flask production run to determine the minipool titre ranking because the static expansion titre data does not correlate to the final bioreactor titre ranking data of the candidate clone.

## Minipool Limiting Dilution:

Limiting dilution is carried out in 40 x 96 well plates using at least 3 minipools to give diversified clones. They first assessed a range of 0.3 to 1 cells per well, but established that 0.5 cells per well was the optimal level.

## Clone Verification and Documentation:

The Solentim Cell Metric imager (Figure 2) is used to image the 40 x 96 well plates on Day 0, Day 1 and Day 2 to monitor the cell division, and again on Day 7 to monitor the colony formation. At this stage, SystImmune identify approximately 200-300 clones with good titre levels, but typically select at least 50 of the top single cell clones for expansion using the Cell Metric image data. They use a very high stringency to determine a single cell image to avoid issues later on when filing their IND.

## Clone Expansion:

Top clones selected from the previous stage (based on image data and titre) are expanded using the same procedure as previously mentioned; expand in static and then move to shake flask. Another 30ml shaker flask fed-batch production run is carried out to identify the top producing clones to move forward.

## Clone Evaluation:

SystImmune decide which clones to move to their Production Department based on cell growth and cell titre assessment, and pass the supernatants to their purification and analytical group to carry out protein quality analysis - CE-SDS (capillary electrophoresis - sodium dodecyl sulfate polyacrylamide gel electrophoresis), HPLC-SEC (high performance liquid chromatography - size exclusion chromatography), cIEF (capillary isoelectric focussing), test binding and function assay. They also generate genomic DNA to monitor copy numbers and test for mycoplasma.

## Production Department:

Eventually SystImmune move the top 8 clones to the Production Department and start 3L bioreactor production runs as well as viable cell density (VCD) and IgG productivity analysis.

## Lead Clone Candidate:

Finally, the top lead candidate is selected and a Research Cell Bank (RCB) is created.

# Challenges Faced in Cell Line Development

Similarly to CLD groups in other companies, SystImmune face several key challenges in their everyday CLD process:

- The complexities of a multistep cell line development process
- Small number of people in the group
- The requirement to reducing timelines and costs
- The need to define success criteria for every step
- The need to document the process for IND filings
- The requirement to demonstrate clonally-derived cell lines


**Figure 2:** The Solentim Cell Metric CLD with incubated plate loader for 10 plates.

With regard to the latter two points, as with many other companies, SystImmune are acutely aware of the FDA's expectations of a clonally-derived cell line which was clearly defined by Dr Sarah Kennett's 'Establishing Clonal Cell Lines – A Regulatory Perspective' presentation. It outlined the stipulation from ICH Q5D that 'For recombinant products, the cell substrate is the transfected cell containing the desired sequences which has been cloned from a single cell progenitor'.

However, achieving clonally-derived commercial production cell lines, and evidence thereof, can be a difficult and onerous task. In the past, many CLD groups have traditionally used two rounds of limiting dilution and theoretical statistical evidence of producing a clonally-derived cell line. However, SystImmune outline that this is a lengthy procedure and observing a final colony at the end does not mean that it originated from a single cell. Also the Poisson distribution used to calculate the probability of clonality does not take into account many factors such as host cell choice, cell behaviour, status and environment which can alter the chances of getting a clonally-derived cell line.

## Documentation of Clonality

SystImmune's chosen path forward to prove clonality was to introduce the Solentim Cell Metric CLD into their cell line development process. The Cell Metric can image the entire well and provide a sharp image of a single cell on Day 0 which is critical proof of a single cell and will allow for a single round of cloning.

In order to verify clonally-derived clones, the figures below demonstrate how SystImmune use the captured images, of both clonal and non-clonal clones, to select their clones to move forward in their process.

In order to provide documentary evidence of clonally-derived cell lines clones, figure 5 below demonstrates how SystImmune produce a Clonality Report using the Cell Metric software. The report generated shows the whole well of interest, the exact location of the clone of interest, how the clone divides and grows over the imaged time points (Day 0 and Day 1 shown here), as well as highlighting any debris in the well that may be confused with cells. Debris, of course, does not grow or divide over the time course and so this evidence is also presented in the report.

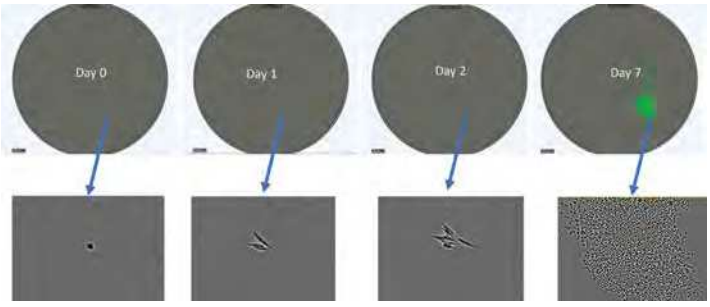



**Figure 3:** Clonally derived cell line example: Whole well imaging at each time point along with zoomed in images of a single cell of interest growing over the time periods into a colony.

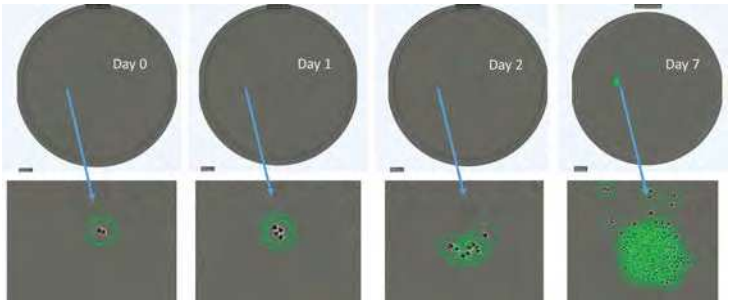



**Figure 4**: Non-clonally derived cell line example: Whole well imaging at each time point along with zoomed in images of two cells (doublet) growing over the time period into a colony.

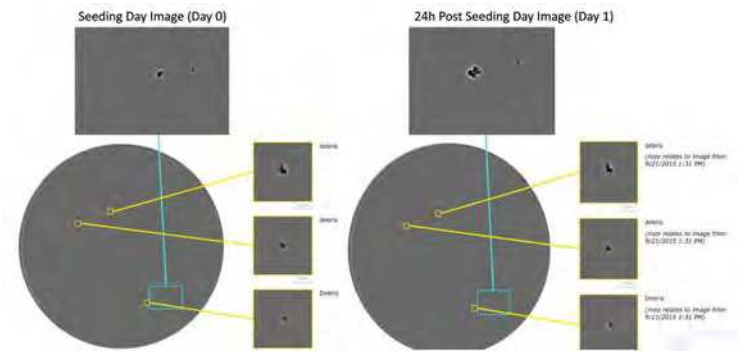
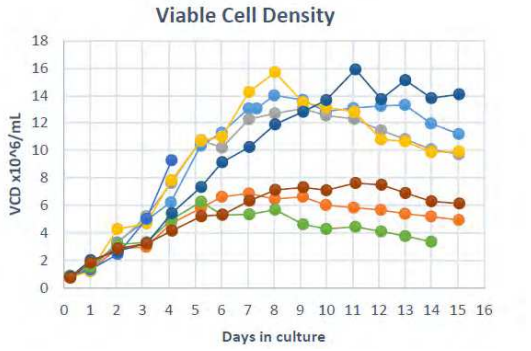


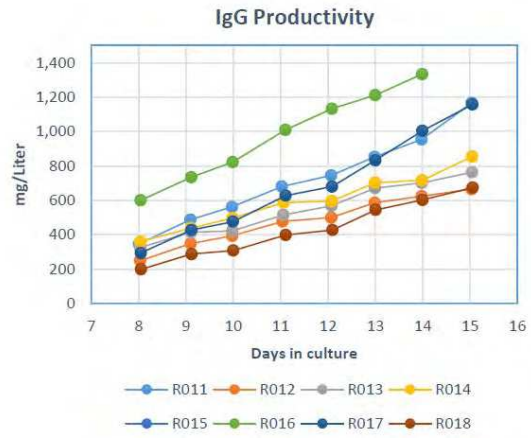

**Figure 5:** Example images taken form a Clonality Report generated by SystImmune on their Cell Metric CLD.

## Productivity Assessment

As outlined previously, 8 of the best clones are passed to the Production Department for bioreactor production runs which are carried out multiple times to analyse viable cell density and IgG productivity.

As evidenced by the graphs, the viable cell density and the protein production over 14 days differs significantly between the 7 clones tested (1 clone was lost). The graph showing productivity highlights three clones that show ≥1 gram per litre on day 14. Interestingly, there is one clone (green – RO16) which has the highest productivity but also has the lowest viable cell density. SystImmune chose this clone as their lead candidate to take forward for further analytical studies.




## Summary

Using the CHOZN Platform and their original cell line development process with two rounds of cloning, it was taking SystImmune approximately 20 weeks to obtain a good lead candidate single cell clone to pass to their production department. The generation of a cell line suitable for production is very time consuming and typically the most rate-limiting step of filing an IND submission.

The demonstration of clonally-derived cell lines is a critical part of the overall regulatory package. With the implementation of both the image capture and the clonality reporting on the Cell Metric, SystImmune are now able to generate evidence of the initial single cell, cell doublings and colony outgrowth.

Using the Cell Metric, SystImmune will eliminate one round of limiting dilution in their CLD process which will save 6-8 weeks of work whilst still meeting the regulatory requirements. This will also be achieved using the existing resource of one FTE in the Cell Line Development group.

## Acknowledgements

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