Pharmaceutical BIOPROCESSING

Toward a two-tier process-development paradigm: prototype versus commercial biomanufacturing

"...a two-tier manufacturing paradigm, addressing separately early prototype versus commercial requirements, could change dramatically how biopharmaceutical development is approached today..."

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Speed is often positioned as a key element in the development of any novel therapeutic. After all, reducing development timelines can have a beneficial impact in managing investment cycles (crucial for small biotech), facilitate the introduction of translational medicine (bench to bedside and back to bench) and extend market exclusivity awarded by intellectual property rights. Yet speed is precisely a largely absent attribute in the development of virtually any new drug, and we could argue that biopharmaceuticals, because of the intrinsic complexities associated with their production, are perhaps the worst positioned amongst most drug classes.

As an optimistic estimate, the road for a lead biopharmaceutical candidate to reach first-in-human clinical trials could take between 1.5 and 2 years of, sometimes perilous, travel involving a considerable out-of-pocket investment at a very high risk. High risk because an immense majority of products in development will fail (sometimes catastrophically) at some point during their development. Below I discuss how past development and manufacturing challenges during the onset of biopharmaceuticals have conditioned the evolution of manufacturing praxis and the perception of risk in the industry. I would also like to discuss how a two-tier manufacturing paradigm, addressing separately early prototype versus commercial requirements, could change dramatically how biopharmaceutical development is approached today, perhaps opening the door to new treatments for medical conditions that today are still largely out-of-reach for protein-based therapeutics, such as infectious diseases.

Who told the first lie? The myth of 'clonality'

Any newcomer to the world of biopharmaceutical development will invariably ask the same question when faced with the prospect of a long and expensive manufacturing development. Is 'clonality' really needed for expression hosts? Those most versed in the art of bioproduction will usually roll their eyes and politely explain that this is a regulatory requirement that all biological products need to comply with, or do they?

Well, strictly speaking, 'commercial' products that are compliant with current Good Manufacturing Practices (cGMP) are usually expected to be produced in a clonal cell line. It is, however, discretionary for regulators to accept a different path for at least some stages of clinical development, according to their assessment of a number of factors, such as the specific characteristics of the target disease, its prevalence, morbidity and mortality risk, the affected patient population, the risk profile for the developed therapeutic and the nature of the treatment envisaged.

But, let us stop for a moment here. Where does this 'clonality requirement' come from? We need to retrace our steps back to the days when biopharmaceuticals made their entrance as the new therapeutic promise to engross the arsenal of treatments to combat human disease.

After Georges Köhler and César Milstein's success in generating hybridomas expressing

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single molecular antibody species in the 70s [1], the whole world of therapeutic monoclonal antibodies became unlocked for exploration, which brought us success stories of the likes of Humira®, Herceptin®, or Avastin®, just to name a few, and even paved the road to more advanced, hybrid therapeutic classes, such as antibody–drug conjugates, which are hailed as one of the future key weapons against cancer and maybe other diseases. Even back in the early days of antibody discovery, the research community was acutely aware of the need to define product identity, in order to be able to establish a correlation with its performance in the clinic. Perhaps inherited from the development of small molecules, it became obvious that clear relationships between chemical and structural identity on one hand, and biological activity in the other, should be determined and controlled as an intrinsic part of biopharmaceutical antibody development. Often this is referred to as structure–activity relationship, or SAR.

Back then, bioprocessing was a far cry from the platforms in existence today. Often, complex biologics, such as monoclonal antibodies, were expressed in hybridomas, requiring complex manipulation and labor-intensive processes. Indeed, some of the products developed in those days are still manufactured in such platforms [2]. Obviously, it was essential to control the identity and 'clonality' of those hybridomas selected for development, as any contamination would invariably produce a mixture of different antibody species, potentially having different biological activities and safety risk profiles.

> "...anyone fluent in cell culture science knows that defining a 'clone' purely in genetic terms is an almost impossible task."

But those days are long gone with the advent of recombinant mammalian expression technologies and the introduction of more versatile cell hosts, like Chinese ovarian hamster (CHO) cells, which are currently the gold standard for mammalian biomanufacturing. Today, genes encoding for a protein of interest are manipulated in complete isolation, optimized to maximize expression and introduced in a 'clean' background cell, therefore producing a single protein sequence.

One might still argue, "What about the complexity and heterogeneity associated with post-translational modifications, particularly glycosylation?" Indeed, it has been shown that monoclonal antibodies expressed in cultures derived from different individual 'clones' (I will discuss this further below) often display variations in their glycosylation profile, which can have an important impact in mode of action, particularly when effector function activity is required for a given therapeutic. However, the advent of biosimilars has shown that the design of the manufacturing process itself has also an important impact in such distribution, often opening the door to reproducing nearly identical (similar) glycoform distributions to originator drugs using even entirely different cell hosts [3] [Flatman S, Boon L, Pers. Comm.].

Furthermore, anyone fluent in cell culture science knows that defining a 'clone' purely in genetic terms is an almost impossible task. Why is this so? Well, for a start biology is never 'static.' It is always 'on the move,' in a continuous change that ultimately underpins biological plasticity and evolution. But in the case of cell lines used in biopharmaceutical expression, like CHO, this plasticity is astonishingly large for any standards [4]. Indeed, CHO cell lines used in bioproduction are known to exhibit significant chromosomal variability [5], even in cases where cultures are derived from a single parental clone [Borth N, Presentation at ESACT-UK (Jan 2014), Unpublished Results], a feature that apparently is shared by other cell types when kept in culture. Nobody really knows what is the impact of such rearrangement in both the expression and chemical and structural properties of a given biopharmaceutical. However, once thing is certain, and it is that, invariably, any cell maintained in culture for a sufficiently long period of time will modify its growth and metabolic patterns and lose its ability to express the intended product of interest. Moreover, it has been shown that individual cells in a culture derived from a single clonal parental cell exhibit a level of variability in their expression patterns that very much matches patterns observed in nonclonal transfectant pools [6], which erodes away any assumed advantage of 'clonal approaches' for bioprocessing.

The science (or rather the 'art') of bioprocessing then is to 'control' as much as possible those variables within an 'acceptable' range by selecting more 'resilient' cell lines and, even more importantly, by defining and controlling culture and process conditions (including time) within a suitable regime that would 'guarantee' that product critical quality attributes (CQAs) are maintained well within required specifications, ideally matched with product biological activity and safety.

It is therefore worth mentioning that the onset of biosimilars has changed radically how CQAs are defined and controlled today. In a relatively quick time, we have moved from a paradigm of 'the product is the process' to a new one in which bioprocessing variables can be harnessed to obtain reproducibly products with the desired characteristics. The introduction of design of experiments methodologies as well as scaled-down bioprocessing models has opened the door to implementing some aspects of quality by design (QbD), although, as discussed elsewhere, significantly more needs to be done in this department [7].

This could well mean the 'triumph' of science and process understanding over empirical observation and qualification by testing. But even if the industry is not yet quite there, it is now clear that product properties can be defined and controlled through process understanding and that they are no longer 'black boxes' that cannot be accessed otherwise.

It is also important to realize that stable 'clonal' hosts are pretty much an 'invention' of the biopharmaceutical industry. We could even argue that it is restricted to the mammalian world, since the immense majority of microbial products make use of plasmids that need to be maintained through stringent selection conditions during culture, and defining 'clonality' in a bacterial host is probably as challenging or more than it is for mammalian cells.

But leaving that aside, there are indeed examples of biotherapeutic products that do not require clonality. For example, virus-based therapeutics including vaccines or gene therapy utilize transient transduction or transfection for the expression of the therapeutic product. So why not biopharmaceuticals?

Is it wise to build a commercial process at a 90% risk of failure?

Still unconvinced? Well, let us now look at the current manufacturing paradigm. In any industry (other than biopharma, of course!), an innovator developing a new product would first build a 'prototype' (somewhere in a 'garage'), so they can properly test its properties, validate the design or identify aspects that would need to be modified or improved. This will be done well before embarking in engineering a manufacturing process or investing heavily in building the required infrastructure or a manufacturing plant. Yet this is exactly the opposite of what happens in biopharma, where pretty much 'commercial' GMP-compliant processes are defined before a product enters clinical trials.

Why? Well, some might claim that this is done to reduce their risk of failure (although most products will fail in development regardless of the level of definition of their commercial manufacturing process), or to increase speed to market (albeit, defining a full commercial process upfront is more likely to slow down progression to the clinic).

If we borrow from current practice in the development of small molecule therapeutics we can see how these arguments do not hold much water. In the small molecule world, new product candidates (prototypes) are often taken into early clinical development using a 'medicinal chemistry process,' that is, a provisional or 'prototype' manufacturing strategy that is able to produce the right molecular species (with acceptable levels of quality and safety) in sufficient quantities for early clinical assessment. And this process will usually need to be redeveloped (sometimes completely revamped) for large scale (commercial) manufacturing to maximize process efficiency, simplify production and ultimately minimize cost of goods. The rationale behind this approach is twofold:

- To allow fast testing of new leads in the clinic and therefore obtain as quickly as possible meaningful safety and efficacy readouts; and
- To minimize costs associated with the development of manufacturing processes by reducing required upfront investments (at risk).

A two-tier process paradigm: prototype versus commercial manufacturing

Following in the footsteps of the 'small molecule world' and current practices used in the manufacturing of viral vaccines and other viral-based therapies we could imagine a situation in which the development of biopharmaceuticals was structured using a two-tier approach:

- Prototype processes: biopharmaceutical lead candidates would be manufactured for early clinical assessment utilizing a simple, well-characterized (prevalidated) process that would not require clonal cell line selection. Such processes could make use for example of transient expression, pooled stable transfectants or pseudoclonal cell lines obtained by means of targeted-integration approaches. Furthermore, candidates could be selected based on their compatibility with a given 'prototype process,' thus minimizing development work and reducing upfront investment, while speeding up the progression of therapeutic candidates to clinical trial validation, and without compromising patient safety! In fact, it is also plausible to assume a scenario in which such 'prototype processes' could be 'prequalified' or 'prevalidated' to address specific quality and safety parameters, regardless of the product expressed. For example, in the case of monoclonal antibodies, candidates could be selected based on their fit to specific predefined platform processes with predefined quality and safety specification outputs;
- Commercial processes: once a given candidate progresses to late stages of development, then a fully fledged commercial process could be implemented. It is also likely that, in the majority of the cases, all the knowledge generated during early development and through the implementation of the prototype process could very well be used to reduce time and

investment required to develop the 'final' commercial processes and increase process predictability.

This paradigm could open the door to new ways of developing biotherapeutics. For example, it could make possible to assess multiple variants of a given therapeutic candidate in the clinic, therefore increasing the level of understanding of their specific mechanisms of action and potential side effects. Moreover, it could also potentially increase the chances of success for novel therapeutic programs in development, which it is ultimately what the pharmaceutical industry is desperately yearning for.

It is also important to note that this approach is fully compatible with the introduction of 'early QbD' methodologies, particularly those aimed to reduce product attrition by mapping specific CQAs in therapeutic candidates during lead selection and lead optimization stages [7,8]. Indeed, these two concepts: two-tier development and early QbD, could be successfully integrated in a new, more efficient, drug development workflow. Interestingly, it has been suggested that nonlinear (nonhierarchical) drug development, involving multiple iterative cycles could be the solution to current shortcomings in R&D productivity for new drugs [9,10].

The future of biomanufacturing? Fast & flexible processes to address unmet medical need

The question that now arises is: could this 'prototype' processes ever become the 'real thing' (as in the 'final process')? For example, can these 'prototype' processes open the door to new approaches to control infectious outbreaks or offer a last-resort therapy to infected patients, particularly in cases of high mortality? There is no shortage of examples of such scenarios, such as the recent Ebola crisis, the risk of a new flu pandemic, MERS outbreaks, polio and TB.

In cases of acute medical and societal need, such an approach could indeed become a credible alternative. For example, in the case of infectious diseases, particularly those with poor medical prognosis or high infectivity, new therapeutic interventions are needed, requiring fast and flexible responses to an outbreak [11]. And let us stress these two aspects: speed and, equally important, flexibility. One can envisage how simpler (but safe) processes could be used to produce therapeutic agents aimed to control infectious outbreaks, ideally in close proximity to the focus of an outbreak.

But could this be extended to other therapeutics? The question to ask is: 'Are we looking at quality in the right context?' To address this issue it is paramount to establish an honest balance between risk over benefit. We would need to assess what would be the potential risk of using such an approach over its expected therapeutic benefit, and compare that to the 'cost' of delaying access to such new therapeutic (in terms of lives, social and economic impact in affected countries) over the safety awarded by a 'well-threaded' slower, more rigid, albeit more predictable path. Having this in mind, in the future such 'fast' and flexible approaches to development might reduce the existing economic barriers to seek effective treatments for still unmet orphan indications, or even perhaps enable the development of novel personalized treatments. Finally we should not forget how such approaches could also facilitate the development of combination therapeutics, for example cocktails of multiple monoclonal antibodies, which seem to show some promise in areas as diverse as oncology or infectious diseases [12,13].

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References

1 Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256(5517), 495–497 (1975).

2 Reichert JM. Marketed therapeutic antibodies compendium. *mAbs* 4(3), 413–415 (2012).

3 Hou JJC, Codamo J, Pilbrough W, Hughes B, Gray PP, Munro TP. New frontiers in cell line development:

challenges for biosimilars. *J. Chem. Technol. Biotechnol.* 86(7), 895–904 (2011).

4 Derouazi M, Martinet D, Besuchet Schmutz N *et al.* Genetic characterization of CHO production host DG44 and derivative recombinant cell lines. *Biochem. Biophys. Res. Commun.* 340(4), 1069–1077 (2006).

5 Cao Y, Kimura S, Itoi T, Honda K, Ohtake H, Omasa T. Construction of BAC-based physical map and analysis of chromosome rearrangement in Chinese hamster ovary cell lines. *Biotechnol. Bioeng.* 109(6), 1357–1367 (2012).

6 Pilbrough W, Munro TP, Gray P. Intraclonal protein expression heterogeneity in recombinant CHO cells. *PLoS ONE* 4(12), e8432 (2009).

7 Zurdo J, Arnell A, Obrezanova O *et al.* Early implementation of QbD in biopharmaceutical development: a practical example. *BioMed Res. Int.* doi:10.1155/2015/605427 (2015) (Epub ahead of print).

8 Zurdo J. Developability assessment as an early de-risking tool for biopharmaceutical development. *Pharm. Bioproc.* 1(1), 29–50 (2013).

9 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? *Nat. Rev. Drug Discov.* 9(11), 856–865 (2010).

10 Fraser J, Kerboul G. A holistic approach to pharmaceutical manufacturing: product lifecycle management support for high yield processes to make safe and effective drugs. *Pharm. Eng.* 32(2), 1–5 (2012).

11 Ebola. A human tragedy and an opportunity for innovation. www.biotechmaverics.wordpress.com

12 Koefoed K, Steinaa L, Soderberg JN *et al.* Rational identification of an optimal antibody mixture for targeting the epidermal growth factor receptor. *mAbs* 3(6), 584–595 (2011).

13 Qiu X, Wong G, Audet J *et al.* Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* 514(7520), 47–53 (2014).