Editorial

Pharmaceutical BIOPROCESSING

Pharm. Bioprocess.
(2013) 1(1), 11–13

Biologics 2.0: can 'omics technology improve mammalian cell-based manufacturing?

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»"There is little doubt that pricing of biologics needs to come under renewed scrutiny to ensure a balance between costs of production and product accessibility. This pressure is also being driven by imminent patent expiry of several high-profile, blockbuster drugs and the emergence of generic biologics."«

Protein-based therapeutics, also known as biologics or biopharmaceuticals, continue to show robust growth in terms of the number of candidates in preclinical development, new approvals and sales of marketed drugs [1,2]. Worldwide sales of biologics exceeded US$100 billion in 2010 and have shown near double-digit growth in the years following. One of the most successful classes of drugs underpinning this growth are monoclonal antibodies [3]. These molecules are complex to produce due their large size and requirement for elaborate post-translational processing to ensure maximal biological activity. Due to these complexities, many biologic therapies are unsustainably expensive, with typical yearly treatment regimes costing from $20,000 to $400,000 [4]. This has placed a huge burden on healthcare systems and has limited availability beyond first-world markets. In addition, it has led to considerable debate on the cost–benefit equation for some of these drugs, which may offer a statistically small life-extension. This is a complex issue as these treatments are often used in cases where no other pharmacological options are available, or to treat rare disease indications. There is little doubt that pricing of biologics needs to come under renewed scrutiny to ensure a balance between cost of production and product accessibility. This pressure is also being driven by imminent patent expiry of several high-profile blockbuster drugs and the emergence of generic biologics – the so-called biosimilars [5]. Established biologics manufacturers and new players will be looking to break into new markets by offering a price-point advantage. It will be intriguing to see if this comes to fruition given the complex regulations around bringing a biosimilar to market.

Regardless of the socioeconomic implications of the above, there is significant interest in reducing the technical development complexities in order to potentially lower the cost of bringing a biologic through early product development. One mechanism that may enable a lower cost is more efficient manufacturing, allowing for greater speed to the clinic while reducing production-cycle times. Most biologics, including monoclonal antibodies, are manufactured by large-scale mammalian cell culture. As an industrial process, mammalian cell-based production of therapeutic proteins has been highly refined over the past 30 years due to pioneering work in controlling the complex bioprocessing parameters, in order to generate high-yielding processes with exquisite batch-to-batch consistency [6,7]. The most commonly used production cell lines in industry today are Chinese hamster ovary (CHO) cells. These cells have predominated large-scale manufacturing due to their established safety record and adaption to robust, high-density suspension growth in bioreactors. While CHO cells have been a very successful host for the biologics industry, significant problems remain. Development of cell lines producing high-quality protein suitable for sustained clinical

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10.4155/PBP.13.9 ISSN 2048-9145

manufacturing is a labor-intensive, time-consuming and a largely heuristic process. In order to create a production cell line, the gene encoding the therapeutic protein must be inserted into the genome of the host and candidates isolated by stringent chemical or metabolic selection. The resulting production levels remain highly variable and product quality issues are unpredictable during early-stage development, and can only be overcome by empirical screening, adding further uncertainty to the process. It is somewhat of a paradox that the underlying issues for producing biologics in mammalian cells such as CHO is their inherent instability and ability to adapt to a range of changing conditions, the very thing that has made them such a successful production host.

There is then an opportunity to make a quantum shift in our ability to control the process of creating mammalian cell production hosts by reducing the black box approach that is currently employed during early-stage development. Moving manufacturing improvements beyond the control of bioprocessing optimization can be thought as the dawn of Biologics 2.0, through the use of a more holistic approach. This has been largely achieved in the microbial world, for the production of next-generation bioproducts, through the use of an integrated systems biology approach [8]. Systems biology as a concept aims to map all conceivable interactions within a system through a set of measurable variables [9]. In the pre-omics era, this was inconceivable, however, data-driven approaches for industrial microbial production processes have delivered increases in production efficiency of over an order of magnitude [10]. The current question for the biologics industry is whether these same techniques and approaches can be applied to considerably more complex mammalian cell systems for the production of high-molecular weight therapeutic proteins [11,12]. Several recent developments look to push this idea towards becoming achievable. First, the acquisition of whole genome sequence data has become possible due to the stunning advances in next-generation deep sequencing [11,13]. This means that gathering real-time gene expression data during biologics development could become a reality leading to a previously unattainable level of cellular readouts. Second, methodologies to both detect and quantify the cellular outputs beyond the transcriptome have rapidly matured. Detailed proteome and metabolome data can be readily generated using latest generation mass spectrometers, among other more traditional techniques [11]. This, coupled with our ability to use more refined metabolic flux analysis, should provide a key link in our ability to interpret these data [14,15]. The next challenge is the development of data-driven *in silico* tools that will enable both integration and interrogation of these data sets in real time [9]. The model for implementation of these analytics would then follow the same quality-by-design principles that are used for current process analytical technology during biologics development.

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The creation of queryable genome scale models for complex eukaryotic systems has made impressive progress over recent years, and there is little doubt we will see this methodology applied during the industrial production of protein therapeutics [16]. The final hurdle will be how these outputs are then applied to re-tool the mammalian production host for more efficient and predictable protein production. This now seems possible due to the advent of a range of precise gene editing tools that are readily available 'off-the-shelf', including zinc finger nucleases, transcription activator-like effector nucleases and advanced homologous recombination, among others [17,18]. These techniques have already been used to create CHO cell lines with altered characteristics based on hypothesis-driven assumptions; however, using the integrated systems biology approach described above will allow a more customized approach to the development of modified production hosts [19]. Within established biopharma there is a wealth of knowledge of molecule 'manufacturability' constraints, which should further aid a rational approach to the development of the next generation of host cells for bioproduction. The challenge will be to create production systems with enhanced properties that provide a more predictable path through development in terms of suitable levels of protein expression, as well as desirable product quality attributes, namely correctly folded molecules with an appropriate post-translational modification profile (e.g., glycosylation, disulfide bond formation and amino acid modifications). It is unlikely that this can be achieved through simple genetic modification, but will more likely

result from a wealth of system-integrated knowledge over time. Where systems biology may have an immediate impact will be modifying core metabolic processes that can be matched with an appropriate feed strategy or replacement of key rate-limiting enzymes with versions that display altered activity and increased energetic flux.

On the surface many of these goals still seem 'blue-sky', however, with the data and information explosion seen over the past 5 years, custom-designed mammalian cell lines are now within reach. Whether this proves to be a truly disruptive technology or merely additive to the significant bioprocess-related milestones already achieved remains to be seen. Regardless, there is significant pressure on biologics developers to bring the next generation of molecules to market with significantly lower price points, which will drive continued investigation of these approaches.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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