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Industry view on the relative importance of "clonality" of biopharmaceutical-producing cell lines

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ABSTRACT

Recently, several health authorities have requested substantial detail from sponsor firms regarding the practices employed to generate the production cell line for recombinant DNA-(rDNA) derived biopharmaceuticals. Two possible inferences from these regulatory agency questions are that (1) assurance of "clonality" of the production cell line is of major importance to assessing the safety and efficacy of the product and (2), without adequate proof of "clonality", additional studies of the cell line and product are often required to further ensure the product's purity and homogeneity. Here we address the topic of "clonality" in the broader context of product quality assurance by current technologies and practices, as well as discuss some of the relevant science and historical perspective. We agree that the clonal derivation of a production cell line is one factor with potential impact, but it is only one of many factors. Further, we believe that regulatory emphasis should be primarily placed on ensuring product quality of the material actually administered to patients, and on ensuring process consistency and implementing appropriate control strategies through the life cycle of the products.

1. Introduction

Recently, several health authorities have requested substantial detail from sponsor firms regarding the practices employed to generate the production cell line for recombinant DNA-(rDNA) derived biopharmaceuticals. Two possible inferences from these regulatory agency questions are that [1] assurance of "clonality" of the production cell line is of major importance to assessing the safety and efficacy of the product and [2], without adequate proof of "clonality", additional studies of the cell line and product are often required to further ensure the product's purity and homogeneity. Here we address the topic of "clonality" in the broader context of product quality assurance by current technologies and practices, as well as discuss some of the relevant science and historical perspective. We agree that the clonal derivation of a production cell line is one factor with potential impact, but it is only one of many factors. Further, we believe that regulatory emphasis should be primarily placed on ensuring product quality of the material actually administered to patients, and on ensuring process consistency and implementing appropriate control strategies through the life cycle of the products.

1.1. Historical perspective

Mammalian cells have been used to produce rDNA-derived human therapeutic proteins for over 25 years. ICH and analogous regional guidelines were developed and orthogonal control strategies have been applied to ensure consistent product safety and efficacy during clinical development and commercialization. These guidelines and control strategies include but are not limited to I) development and validation of appropriate and robust

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manufacturing processes, starting with generation of the production cell line and II) monitoring of those manufacturing processes and resulting biopharmaceutical products through pertinent control strategies and characterization. Collectively, these control principles have worked well to provide a steady stream of over 100 therapeutic biopharmaceuticals which have significantly benefited public health, while building an admirable record from the standpoint of product consistency and safety over this period of time [1,2].

An important characteristic of any biopharmaceutical manufacturing process is consistent cell culture performance, in turn delivering consistency in product quality attributes. Cell culture performance can be impacted by many factors, including the production cell line. However, it is critical to recognize that a culture of any production cell line consists of a population of cells and absolute genetic homogeneity, whether of the transgene or at the genomic level, is not achievable given the genomic plasticity inherent to immortalized mammalian cell lines [3]. This situation is especially true in the case of immortalized cell lines typically employed for production (e.g. CHO and NS0) and the generational span encompassed from introduction of product-encoding transgene to the End of Production Cells at the limit for *in vitro* cell age [4]. Therefore, referring to a production cell line as a "clone" or to the "clonality" of a manufacturing cell bank is misleading, as any population of these types of cells cultured for a length of time will accumulate genetic and phenotypic heterogeneity [5]. In the strictest sense, a more accurate description would be that these cell lines can have a high probability of being clonally-derived. That is, the cells can be grown from what is likely to be a single cell through a laboratory manipulation (termed "cloning"). One can subsequently obtain a population of cells that are derived from that cloning event. Post-cloning, during cell growth and expansion, a variety of factors create genomic heterogeneity including inherent DNA replication errors, error-prone SOS DNA repair processes of immortal cell lines and Darwinian selection. These factors combine to introduce, amplify, and select for genetic variation within the cell population. To better understand the potential impact of these types of cells on biopharmaceutical manufacturing, it is important to first understand their underlying nature.

The ability to grow mammalian cells for rDNA technology in culture has relied on the selection of cell populations that have escaped normal control of cell division. This attribute is inherently aided by genetic perturbations that cause impairment of cell cycle checkpoints, and early attempts to grow cells in culture for extended periods were only successful when they were isolated from neoplastic tissue or were spontaneously "transformed". Many cell lines generated in this way displayed increased genetic drift and chromosomal instability. For the biotechnology industry, this research fortuitously involved the isolation of an immortalized cell line from an ovarian biopsy of a juvenile female Chinese hamster [6,7]. This work was part of much broader studies in which careful techniques were honed to isolate human cell lines from a variety of tissues. It would have never been imagined by Theodore Puck and others that these hamster-derived cell lines would go on to become the production system underlying our ability to produce a multitude of protein therapeutics which have changed the lives of so many patients and helped create a biotechnology revolution.

The commercial need to grow mammalian cells outpaced the fundamental scientific understanding and for many years successfully culturing these cells relied on the presence of complex, undefined additives such as serum or embryo extracts. Advancement in bioreactor engineering, characterization, automation, and the development of chemically-defined media in addition to the ability to adapt cells to suspension growth has enabled the biotechnology industry to expand cells rapidly from a cryopreserved state into culture volumes ranging from static microwell plate with culture volumes of <1 mL to >20,000 L in stirred bioreactors. Despite these advances, the fundamental nature of these cells do not allow for control of the genetic and phenotypic drifts that occur whenever such mammalian cells are grown in culture. Though this genetic drift can present potential challenges, it is important to place this in context as these inherent characteristics underpin the ability of these cells to accept transgenes and to adapt readily to process conditions. For example, it allows adaptation of cells to a variety of basal culture media and growth under demanding process conditions. These changes occur at an individual cell level within a population even within clonally-derived populations [5]. This point is fundamentally important as it underscores the potential genetic and phenotypic changes that occur within a cell population irrespective of the origin of the cells.

It is also important to recognize the complex and varied origin of the cell lines used within industry and the inherent genetic perturbations that are present. For example, the commonly used dihydrofolate reductase (DHFR) deficient DXB11 and DG44 CHO cell lines were generated via multiple rounds of chemical and radiation-induced mutagenesis [8,9]. This has resulted in CHO cell lines whose karyotypes are very different from that of the parental hamster. A recently published CHO cell line karyotype demonstrated that, in contrast to the 22-chromosome diploid genome of the hamster, even the non-mutagenized CHO-K1 cell line had only 21 chromosomes, of which only eight were cytogenetically similar to the hamster and the remaining 13 showed extensive changes via deletions, reciprocal and nonreciprocal translocations and pericentric inversions [10]. The results of research in this area clearly demonstrate the genetic plasticity that occurs when these types of cell lines are cultured over time, and provides additional technical justification that the primary focus for any biopharmaceutical manufacturing process should be on the product being produced rather than on the "clonality assurance" of the cell line used for its derivation. Similarly, the product is ultimately highly purified, and the focus should be on product and process consistency rather than on uncontrollable aspects of the cell lines used to produce the product. As will be discussed in more detail later, it is also important to bear in mind that the product, not the cells, is administered to patients.

In spite of the perceived negative view of the genetic plasticity inherent to these types of cells, it is a critical factor that the biopharmaceutical industry has been dependent upon for the past three decades. Cellular genetic plasticity fundamentally underpins our ability to genetically engineer cell lines to be an appropriate substrate for biopharmaceutical manufacturing and improve their performance by directly impacting productivity and product quality. A cornerstone of the biotechnology industry has been the ability to readily insert a foreign transgene into the host cell genome and expect a reasonable number of cells to accommodate this manipulation. Transgene incorporation relies on a method to facilitate expression plasmid delivery, typically via electroporation or lipid-mediated reagents, and subsequent random integration of the plasmid DNA into the genome. The use of random integration as the current standard approach results in significant heterogeneity in which the transgene location within a cell will vary across the selected population. The level of genetic heterogeneity is further complicated by the methods employed to select for cells which have integrated the transgene and which produce acceptable yields of protein product. Within industry these methods typically rely on the use of metabolic markers as selective pressure in which the plasmid encodes a gene which complements a deficiency of the cell line used, typically DHFR or glutamine synthetase (GS) mutant CHO cells under conditions where expression of those genes is favored

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