# Producing recombinant proteins for human use

Chinese Hamster Ovary (CHO) cell lines are the most frequently used cell lines in the biopharmaceutical industry for the production of therapeutic recombinant proteins, because they can be easily genetically manipulated, grow quickly, can be easily adapted to serum-free conditions, and are able to produce glycosylated proteins in large amounts with low risk of transmitting human pathogenic viruses. However, they include many sub-lineages which are genetically heterogeneous, and are prone to chromosomal rearrangements and gene loss during successive generations and gene amplification. Therefore, CHO cell lines used for bio-therapeutics production should be clonally derived, and all cells within a given clonal cell population should be genetically and phenotypically (morphology, behavior, etc.) identical. Indeed, the genetic stability of mammalian cell lines expressing therapeutic proteins is crucial for maintaining a consistent bio-production to guarantee the product identity, potency, quality and purity, thus ensuring its safety and efficacy.

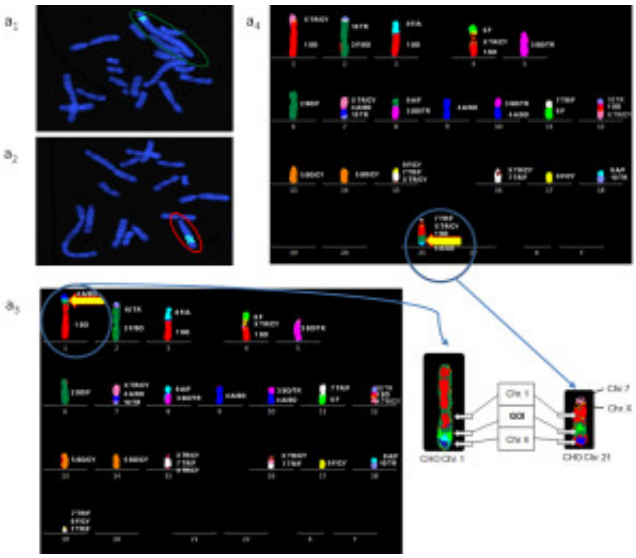

Fig. 1. (from PDA J Pharm Sci and Tech 2015, 69, 540-552): a1, a2: FISH analysis. a1) green circle: GOI expected location on the longest chromosome; a2) red circle: GOI unexpected location on a shorter chromosome; a3-a4: MCP analyses: a3: normal karyotype (GOI: yellow arrow) on CHO chromosome 1; a4: unexpected karyotype (GOI: yellow arrow) on novel derivative CHO chromosome at position 21

Before being used for commercial production, cell lines are submitted to a qualification program in order to ensure their phenotypic and genotypic characteristics and the product efficacy and safety. During the production life cycle of a therapeutic protein, additional cell banks have to be validated after exhaustion of the current qualified cell bank, in order to support the commercial production

continuum of the recombinant protein. It is during the validation of an additional cell bank derived from a validated cell bank, that we detected in a small portion of cells at the end of the production phase in bioreactor, a different chromosome bearing the gene of interest (GOI). The genetic aberration was identified by fluorescence in situ hybridization (FISH), a cytogenetic technology allowing the identification of the position of specific DNA sequences on chromosomes. While the GOI is normally located on the longest chromosome, it was situated on a shorter chromosome in these cells (Fig. 1 $a_1$-$a_2$). Additional analyses were performed, using multicolor chromosome painting (MCP), a cytogenetic method enabling the identification of both numerical and structural chromosomal aberrations (Fig. 1 $a_3$-$a_4$). MCP indicated that the unexpected location of the GOI occurred as a result of a reciprocal chromosomal translocation. Whereas in the normal karyotype (complete set of chromosomes), the GOI is located close to the end of the CHO chromosome 1 and adjacent to hamster-derived chromosome 1 and 4 regions, in the unexpected karyotype, the GOI was located close to the end of a derivative CHO chromosome at position 21, bearing a chromosome 7 region followed by a short chromosome X region. The derivative chromosome displayed the same chromosome 1 and chromosome 4 flanking (adjacent to the gene) regions of the original integration site, indicating that the insert is not affected in its integrity.

In our case, cells bearing the translocation did not affect process performance and product quality. However, this event highlights the need to characterize the integrity of the GOI in end-of-production cells when producing recombinant proteins for human use, because in case of chromosomal aberrations, there is a risk that some changes occur in cell behavior which might negatively impact process performance, product quality and safety.

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## Publication

Reciprocal Translocation Observed in End-of-Production Cells of a Commercial CHO-Based Process.
Rouiller Y, Kleuser B, Toso E, Palinksy W, Rossi M, Rossatto P, Barberio D, Broly H
*PDA J Pharm Sci Technol. 2015 Jul-Aug*