Dev Immunol 245181), sarcomas and thyroid carcinomas (Blanco et al., 2013, Journal of Biomarkers, 602417) and malignancies from the digestive (Blanco et al., 2011, ISRN Gastroenterol 645641) and genitourinary (Blanco et al., 2011, ISRN Pathology, 953803) systems.

14F7 mAb is able to kill tumor cells expressing the ganglioside in a complement-independent manner (Carr et al., 2002, Hybrid Hybridomics 21, 463-468; Roque-Navarro et al., 2008, Mol Cancer Ther 7, 2033-2041). A humanized version of this antibody, obtained by the modification of potential human T cell epitopes (Patent application No. 200480017457.8; Mateo et al., 2000, Hybridoma 19, 463-471) and named 14F7h, retained the properties of the mouse and chimeric antibodies (Fernandez-Marrero et al., 2011, Immunobiology 216, 1239-1247). Humanized 14F7hmAb is of potential value for the therapy of GM3(NeuGc) -expressing tumors.

However recombinant NS0 myeloma cell lines expressing antibody 14F7h lost viability in high density cell cultures because these cells expressed the antigen GM3(Neu5Gc) and were killed by the secreted cytotoxic antibody. Therefore the recombinant humanized antibody 14F7h was expressed in murine NS0 myeloma cell line defective in N-glycolylated-glycoconjugates, due to the knock down of the CMP-N-acetylneuraminic acid hydroxylase enzyme (Fernandez-Marrero et al., 2011, Immunobiology 216, 1239-1247). Such cell line showed to be very difficult to adapt to grow in serum-free medium.

In the present invention we have established a new approach to develop stable high producer cell clones to be used in perfusion fermentation production process. Following this approach cell clones were recovered from a recombinant NS0 myeloma cell line which produces an anti-GM3(NeuGc) monoclonal antibody. Identity attributes of such therapeutic antibody are also disclosed in this invention, which define a molecular phenotype for this biopharmaceutical.

SUMMARY

In one aspect, the present invention refers to a method for obtaining stable high producer cell clones from NS0 recombinant myeloma cell lines in protein-free medium producing humanized NeuGcGM3 antibody 14F7h for industrial purposes that comprise three stages:

I. Adaptation to protein-free medium by a stepwise reduction of Cell Boost supplement to protein-free chemical medium in low density stationary cell culture
II. Adaptation to protein-free medium in high density cell culture using perfusion fermentation system in 5 L bioreactor at lab scale
III. Selection of stable high producer cell clones from cells at the end of fermentation; wherein said stable high producer cell clones have a growth specific rate (μ) higher than 0.025 h^{-1} and a production specific rate (QP) higher than 0.18 pg/cell*h when measured after 30, 60 or 90 days of continuous cell culture.

CLAIMS

1. A method for obtaining stable high producer cell clones from NS0 recombinant myeloma cell lines in protein-free medium producing humanized NeuGcGM3 antibody 14F7h for industrial purposes that comprise three stages:

I. Adaptation to protein-free medium by a stepwise reduction of Cell Boost supplement to protein-free chemical medium in low density stationary cell culture

II. Adaptation to protein-free medium in high density cell culture using perfusion fermentation system in 5 L bioreactor at lab scale

III. Selection of stable high producer cell clones from cells at the end of fermentation; wherein said stable high producer cell clones have a growth specific rate (μ) higher than 0.025 h^{-1} and a production specific rate (QP) higher than 0.18 pg/cell*h when measured after 30, 60 or 90 days of continuous cell culture.

2. The method of claim 1 wherein the first stage comprises the following steps:

I. Adaptation to grow in PFHMII cell culture medium + 3,5 g/L Cell Boost. Serial passage is performed until maximal cell concentration (Xv) reaches a constant value.

II. Adaptation to grow in PFHMII cell culture medium + 1 g/L Cell Boost. Serial passage is performed until Xv reaches a constant value.

III. Adaptation to grow in PFHMII cell culture medium. Cells are allowed to grow in protein-free medium without any supplement for 60 days.

3. The method of claim 1 wherein the second stage comprises the following steps:

I. Adaptation to grow in protein-free PFHMII cell culture medium at high cell density using perfusion fermentation system in 5 L bioreactor for more than 21 days, wherein said high cell density is at least 5-10 x 10^6 cells/ml.

II. Cells at the end of fermentation are frozen and stored in liquid nitrogen (that is end production cell bank, EPCB).

4. The method of claim 1 wherein the third stage comprises the following steps:

I. Defrosted cells from EPCB are subjected to cell cloning by the limiting dilution method;

II. Antibody-secreting clones are detected by a highly sensitive and specific ELISA method using an anti-idiotypic antibody as capture antigen;

III. Quantification of the antibody-producer cell sub-population in selected clones by FACS using intracellular immunoglobulin staining;

IV. Kinetic studies to select clones with a specific growth rate (μ) higher than 0.025 h^{-1} and a production specific rate (QP) higher than 0.18 pg/cell*h when measured after 30, 60 or 90 days of continuous cell culture.